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NEW SPECIES, VARIETIES AND COMBINATIONS FROM THE HERBARIUM AND THE COLLECTIONS OF THE ARNOLD ARBORETUM[1]

ALFRED REHDER

With nine text figures

Celtis Rockii, sp. nov.

Arbor 6-metralis, ramulis hornotinis dense flave crispulo-villosis angulatis vel subangulatis lenticellatis, annotinis tarde glabrescentibus; gemmae ovoideae, acutae, perulis adpresse flavo-pilosis. Folia papyracea, elliptico- vel rhombico-ovata, 4-8 cm. longa et 2.3-4.5 cm. lata, breviter acuminata, basi plus minusve oblique late cuneata vel fere rotundata, a medio vel infra medium ad apicem dentato-serrata, supra accumbenti-pilosa et scabrida, subtus tota facie satis dense molliter crispulo-villosa, ad nervos pilis longioribus patentibus flavidis instructa, triplinervia, nervis utrinsecus basalibus inclusis 2-3; petioli flave crispulo-villosi, 3-5 mm. longi. Flores non visi. Racemi fructiferi axillares in parte inferiore ramulorum plerique triflori, pedicello terminali incluso 1-1.5 cm. longi, dense flave crispulo-villosi, pedunculo 2-5, pedicellis 3-8 mm. longis; drupa subglobosa, parva, plus minusve villosula, vel maturitate glabra vel fere glabra, lutea (ex collectore) in sicco atro-fusca; putamen subglobosum, circ. 4 mm. diam., manifeste punctulato-foveolatum et leviter costatum.

CHINA. Yunnan: region of Tungshan, Yangtze drainage basin, east of Likiang, *J. F. Rock,* no. 10522 (type), in 1923 (tree 20 ft.; fruits yellow).

This new species seems closely related to *C. Salvatiana* Schneid., and also to *C. cinnamomea* Lindl., but from both it is easily distinguished by the rather dense pubescence of the leaves, the densely pubescent branchlets and inflorescence and the pubescent fruit; from *C. cinnamomea* it differs further in the serrate broader leaves, the few-flowered short-peduncled inflorescence and smaller fruit.

[1]Continued from vol. XIII. 341.

Clematis chinensis Osbeck, Dagb. Ostind. Resa, 205, 242 (1757); Reise Ostind. China, 267, 315 (1765); Voy. China East Ind. I. 329; II. 356 (1771).—Retzius, Observ. II. 18, t. 2 (1781).—De Candolle, Syst. I. 137 (1818); Prodr. I. 3 (1824).—Forbes in Jour. Bot. XXII. 262 (1884).—Hemsley in Jour. Linn. Soc. XXIII. 3 (1886).—Pritzel in Bot. Jahrb. XXIX. 332 (1900).—Finet & Gagnepain in Bull. Soc. Bot. France, L. 535 (1903); Contrib. Fl. As. Or. I. 20 (1905), excl. synon. *C. terniflora* et *C. Benthamiana*.—Rehder & Wilson in Sargent, Pl. Wilson. I. 329 (1913); in Jour. Arnold Arb. VIII. 106 (1927).—Léveillé, Fl. Kouy-Tchéou, 332 (1915).—Merrill in Jour. Am. Bot. III. 579 (1916).—Rehder in Jour. Arnold Arb. X. 187 (1929).

Clematis sinensis Loureiro, Fl. Cochinch. 345 (1790).

Clematis minor Loureiro, l. c. (1790).—De Candolle, Syst. I. 136 (1818); Prodr. I. 3 (1824).—Forbes in Jour. Bot. XXII. 263 (1884).

Clematis recta § *chinensis* Kuntze in Verh. Bot. Ver. Brandenb. XXVI. 114 (Monog. Clem.) (1885).

Clematis funebris Léveillé & Vaniot in Bull. Acad. Intern. Géog. Bot. XI. 168 (1902).

Clematis oligocarpa Léveillé & Vaniot, l. c. XVII. no. 210-11, p. ii (1907).—Léveillé, Fl. Kouy-Tchéou, 333 (1915).

Clematis Cavaleriei Léveillé & Porter in Fedde Rep. Spec. Nov. IX. 20 (1910).—Léveillé, Fl. Kouy-Tchéou, 332 (1915).

The fact that the generally accepted name *Clematis chinensis* Retzius would be invalidated by the older homonym *C. chinensis* Osbeck, if the two names referred to different species, has led me to investigate this question and I find that the two names are synonymous, as they have been already treated by a few authors. This will save the specific epithet *chinensis* for the species generally known under this name, and makes necessary the change of the author citation only, so that it will be *C. chinensis* Osbeck (1757) instead of Retzius (1781).

Owing to the fact that Osbeck's name was published with a rather incomplete description hidden away in the text of a work not primarily taxonomic, it has been neglected, while Retzius' name published with an adequate description accompanied by a plate has been universally accepted. Also the misleading citation in Index kewensis of *C. chinensis* Osbeck as a synonym of *C. recta* L. may have caused the disregard of the name by later authors. Moreover E. D. Merrill (l. c.) has tried to identify *C. chinensis* Retz. with *C. Meyeniana*, but the description given by Osbeck (l. c. p. 205) "Plurima habet communia Clematide Vitalba, at folia lanceolata, angustissima, & flores minores" agrees much better with *C. chinensis* Retz. which has pinnate leaves like *C. Vitalba* with narrower leaflets and decidedly smaller flowers, while *C. Meyeni-*

ana has ternate leaves with large subcoriaceous leaflets, quite different from the pinnate leaves of *C. Vitalba*, and flowers scarcely smaller than those of the latter species. Additional data given by Osbeck (l. c. p. 242) "Pistilla 3 ad 6, stylis plumosis in orbem positis reflexis. Stam. O obseruavi. Frutex scandens, ramosissimus" may apply as well to *C. chinensis* as to *C. Meyeniana*. In his more recent Commentary on Loureiro's Flora cochinchinensis (msc.), however, Merrill identifies *Clematis minor* Lour. with *C. chinensis* Osbeck and states that according to his opinion *C. chinensis* Retz. and *C. chinensis* Osbeck are identical; he also considers *C. Benthamiana* Hemsl. a synonym, following Finet & Gagnepain, which I keep separate (see Rehder & Wilson, l. c.).

The identity of *C. chinensis* of Osbeck and of Retzius is proven conclusively by specimens before me, for the loan of which I am indebted to those in charge of the herbarium of the State Museum in Stockholm. One of these specimens is marked on the back of the sheet "China: Osbeck," a second "Herb. Swartzii—Osb." and a third "ex Ind. Orient.," the last note may have reference to the fact that it was collected during Osbeck's East Indian voyages, but as the species does not occur in East India, the specimen probably came from China. All these specimens represent very early collections and were probably all collected by Osbeck; they are all named *Clematis chinensis* Retz., the determinations being apparently of a later date, for two of them bear the citation "DC." The specimens vary somewhat in the shape and size of the leaves and only one has leaflets as narrow and small as in Retzius' plate of *C. chinensis*.

Clematis chinensis is widely distributed in southeastern and central China extending west to Szechuan and Kweichou. In this herbarium there are specimens from the following provinces: Kwangtung, Fukien, Hunan, Anhwei, Chekiang, Kiangsi, Hupeh, Szechuan and Kweichou; there is also a specimen from Annam, and a photograph of the type of *C. minor* Lour. from Cochinchina.

Clematis grata Wall. var. **likiangensis**, var. nov.

A typo recedit achaeniis glabris.—Ramuli, petioli et inflorescentiae laxe villosula. Foliola ovata, trilobata lobis grosse paucidentatis, supra glabrescentia, subtus in costa venisque densius, in venulis sparsius et in facie sparsissime flavido-pilosis; flores ut in typo carpellis glabris exceptis.

China. Yunnan: Yangtze watershed, Prefectural district of Likiang, eastern slopes of Likiang Snow Range, *J. F. Rock*, nos. 3668 (type) and 3918.

Clematis grata apparently varies like the related *C. Gouriana* Roxb.,

and *C. brevicaudata* DC. with pubescent and glabrous akenes (*C. Gouriana* var. *Finetii* Rehd. & Wils. and *C. brevicaudata* var. *lissocarpa* and var. *subsericea* Rehd. & Wils.), which shows that the pubescence of the akenes is a character of secondary importance and cannot be used to define subdivisions of the genus.

Deutzia Esquirolii (Lévl.), comb. nov.

Styrax Esquirolii Léveillé in Fedde Rep. Spec. Nov. IX. 446 (1911).

Deutzia lancifolia Rehder in Sargent, Pl. Wilson. I. 147 (1912); Jour. Arnold Arb. XII. 276 (1931).—Léveillé, Fl. Kouy-Tchéou, 387 (1915).

Deutzia Chaffanjoni Léveillé, l. c. (1915), pro synon. *D. lancifoliae* Rehd.

Deutzia Esquirolii (Lévl.) Léveillé, l. c. (1915), pro synon. *D. lancifoliae* Rehd.

When dealing with *D. lancifolia* in my Notes on the ligneous plants described by Léveillé (Jour. Arnold Arb. XII. 276) I overlooked that unfortunately Léveillé's *Styrax Esquirolii* is one year older than my *Deutzia lancifolia* and that the new combination resulting from the transfer of the specific epithet of his name should be the valid name for the species. Léveillé himself had already published this combination, but only as a synonym of *D. lancifolia.*

Hydrangea umbellata Rehder in Sargent, Pl. Wilson. I. 25 (1911).

Hydrangea Schindleri Engler in Engler & Prantl, Nat. Pflanzenfam. ed. 2, XVIII-A, 203 (1930), pro parte.—**Synon. nov.**

Hydrangea Schindleri was only briefly mentioned in Engler's account of the species of *Hydrangea* (l. c.) without enumeration of specimens. Engler compared it with *H. chinensis* Maxim. and *H. umbellata* Rehd. In the Berlin Herbarium there are four specimens labeled *H. Schindleri* all collected by A. K. Schindler in August-September 1908 at Lu-shan, Kuling mountains, Kiangsi, the type locality of *H. umbellata.* Two of them, nos. 325 and 327, I cannot distinguish from *H. umbellata* while the other two numbers belong to the following species:

Hydrangea paniculata Siebold in Nov. Act. Acad. Leop.-Carol. XIV. pt. II. 690 (Syn. Hydr.) (1829).

Hydrangea Schindleri Engler in Engler & Prantl, Nat. Pflanzenfam. ed. 2, XVIII-A, 203 (1930), pro parte.— **Synon. nov.**

Of the four numbers collected by Schindler at Lu-shan, Kiangsi, and named by A. Engler *H. Schindleri* two belong to the preceding species, while the other two, nos. 322a and 324, are identical with *H. paniculata* Sieb. No. 324 bears the following note in A. Engler's handwriting, "Hydrangea Schindleri Engl. n. sp., affinis Hydr. chinensi Maxim., differt foliis ab infima triente sursum angustatis, haud e medio utrinque angustatis, distinctius serratis, florum sterilium sepalis ovatis angusti-

oribus." This seems to show that this specimen should be considered the type of *H. Schindleri* Engl., since the preceding characterization is apparently based on Schindler's no. 324 rather than on any of the other numbers and is the same as given in German in the Pflanzenfamilien.

In 1911 in my Synopsis of the Chinese species of Hydrangea (in Sargent, Pl. Wilson, I. 25, 1911) I stated that Wilson's no. 1601, collected at Kuling, July 27, 1907, was to my knowledge the first specimen of *H. paniculata* collected in China. Since then, however, many additional specimens have come to this herbarium and the species is now known from the following Chinese provinces: Kiangsu: Yii-du-hsien, *H. H. Hu*, no. 1179. Anhwei: Chu-hwa-shan, *R. C. Ching*, no. 2808; Wu-yen, *N. K. Ip*, no. 7675. Chekiang: Tsing-Tien, Taishun-hsien and Chang-shan-hsien, *Y. L. Keng*, nos. 172, 310 and 841; Pang-yung, *R. C. Ching*, no. 2099; East Tien-mu, *H. H. Hu*, no. 1609. Kiangsi: Kuling, *E. H. Wilson*, no. 1601; Lu-shan (Kuling), *A. K. Schindler*, nos. 322a, 324; Lu-shan, *A. N. Steward*, no. 2613; Ningdu, *Wang-Te-Hui* in Handel-Mazzetti, Pl. Sin., no. 442. Fukien: Yenping, *H. H. Chung*, nos. 2844, 3301, 3556 and 3659. Kwangtung: Lokchong, *Y. Tsiang*, no. 1219; between Bei-shen and Nan-shung, *W. Y. Chun*, no. 5683; road to Chang-kiang, *W. Y. Chung*, no. 5794; Siudsao, *R. Mell*, no. 1773. Hunan: Wukang, *Handel-Mazzetti*, no. 12527. Kweichou: Lou-tsong-koan, *E. Bodinier*, no. 1661; Kwei-yang, *Handel-Mazzetti*, no. 10478; Kweiting, *Y. Tsiang*, no. 5627. Yunnan: Yunnanfu, *O. Schoch*, no. 423. The specimen collected in 1897 by E. Bodinier in Kweichou was described by Léveillé as *H. Kamienskii* (cf. Jour. Arnold Arb. XII. 277).

Spiraea yunnanensis Franchet, Pl. Delavay. 200 (1890).—Schneider, Ill. Handl. Laubholzk. I. 463 (1905).

Spiraea sinobrahuica W. W. Smith in Not. Bot. Gard. Edinb. x. 67 (1917); XIV. 233, 260 (1924); XVII. 388 (1930).—**Synon. nov.**

Spiraea sinobrahuica var. *aridicola* W. W. Smith in Not. Bot. Gard. Edinb. x. 68 (1917); XVII. 197, 363 (1930).—**Synon. nov.**

CHINA. Szechuan: in valle fl. Ming, inter stationes Sim-puguanj et Pei-schuy-tchan, *G. N. Potanin*, Aug. 25, 1873 (frutex usque metralis); inter Tatsien-lu et Batang, ad stationem Natschuka sive Nachtschuka, *V. Kashkarov*, May 19, 1893 (frutex plus quam metralis); between Batang and Tschien-lu, *John R. Muir*, in 1911; Muli kingdom, Shou-chu valley, alt. 2435-2900 m., *J. F. Rock*, no. 16279, June 1928. (shrub 1-1.5 m.) Yunnan: terrains calcaires, pierreux au dessus de Mo-so-yn, Lankong, alt. 2200 m. *J. Delavay*, no. 1082, (holotype of *S. yunnanensis*, photo. and fragments in A. A.), May 1,

1884 (arbrisseau d'un mètre; fleurs blanches); eastern flank of the Lichiang range, Lat. 27° 10′ N., alt. 9000-10500 ft., amongst the scrub in side valleys, *G. Forrest, no.* 5580 (syntype of *S. sinobrahuica;* ex W. W. Smith, l. c.); descent of the Yangtze from the eastern boundary of the Lichiang valley, lat. 27° 15′ N., alt. 9000-10000 ft., *G. Forrest,* no. 10117 (syntype of *S. sinobrahuica*), June 1913 (shrub 4-5 ft.; flowers creamy white); descent of the Yangtze valley from the eastern range of the Lichiang valley, Lat. 27° 30′ N., alt. 9000-10000 ft., *G. Forrest,* no. 10084 (syntype of *S. sinobrahuica* var. *aridicola*), June 1913 (shrub 4-6 ft.; flowers creamy white); mountains of Chungtien plateau, lat. 27° 30′ N., alt. 11000 ft., *G. Forrest,* no. 12634 (syntype of *S. sinobrachuica* var. *aridicola;* photo. in A. A.); open stony slopes and on ledges of dry cliffs on the western flank of the Lichiang range, Lat. 27° 40′ N., Long. 100° 18′ E., alt. 10-11000 ft., *G. Forrest,* no. 21171, May 1922 (shrub 3-5 ft.; flowers creamy-white); Yangtze valley, northwest of Likiang, Lat. 27° 20-30′ N.; alt. 2000-2100 m., *Handel-Mazzetti,* no. 8792, June 2, 1916; Yangtze watershed, Prefectural district of Likiang, eastern slopes of Likiang snow range, *J. F. Rock,* no. 3639, May-Oct. 1922; western slope of Likiang snow range, Yangtze watershed, *J. F. Rock,* no. 8557, April 1923 (shrub forming globose bushes); Lotueshan, mountains of Labako, west of Yangtze bend at Shiku, *J. F. Rock,* no. 8471, April 1923 (shrub 3-4 ft.; flowers white); dry rocky slopes and on cliffs on the Chien-chuan-Mekong divide, Lat. 26° 36′ N., Long. 99° 40′ E., alt. 9-10000 ft., *G. Forrest,* no. 21465, July 1922 (shrub of 2-3 ft.; flowers white); open dry rocky slopes and ledges of cliffs on the Mekong-Salween divide, Lat. 28° 12′ N.; alt. 7-9000 ft., *G. Forrest,* no. 16410, May 1918 (shrub 1-3 ft., flowers creamy white). Southeastern Tibet: Tsarong, open stony situations on the ledges of cliffs on the Salween-Kiu-chiang divide, Lat. 28° 40′ N., alt. 7000 ft., *G. Forrest,* no. 18881, July 1919; on ledges and in crevices of cliffs and dry bouldery slopes on the Salween-Kiu-chiang divide, Lat. 28° 40′ N., Long. 98° 15′ E., alt. 8000 ft., *G. Forrest,* no. 19147, Sept. 1919 (shrub 1-2 ft., widely branched).

I have been unable to find any characters to separate *Spiraea sinobrahuica* W. W. Sm. from *S. yunnanensis* Franch. The chief difference given by the author of the former name, the glabrous upper surface of the leaves of *S. yunnanensis,* does not hold, since the leaves of the type specimen of *S. yunnanensis* show the same pubescence of short accumbent hairs on their upper surface as the types of *S. sinobrahuica,* though Franchet describes them as glabrous, a statement which induced the author of *S. sinobrahuica* to consider Forrest's specimens distinct. It also does not seem possible to separate var. *aridicola* from the type.

The species which is closely related to *S. brahuica* Boiss. is very variable in the size, in the serration and to some extent in the shape of the leaves, in the size of inflorescence and in habit; the two extremes merge imperceptibly into each other and are apparently only individual differences caused by difference of exposure and soil. The only form which seems worthy of a distinct name on account of its striking habit is the following.

Spiraea yunnanensis f. **tortuosa** (Rehd.), forma nova.

Spiraea tortuosa Rehder in Sargent, Pl. Wilson. 1. 445 (1913).

CHINA. S z e c h u a n : Mao-chou, arid regions of the Min valley, alt., 13-2000 m., *E. H. Wilson,* no. 2764 (holotype of *S. tortuosa*), May 25, 1908 (shrub 3-4 ft.; flowers white).

This differs strikingly from the type in its distinctly zigzag branchlets, the perfectly straight internodes forming sharp angles of about 130-150° with each other; the leaves are suborbicular to broadly oval, more or less 3-lobed and scarcely exceed 12 mm. in length; the inflorescence is 5-12-flowered. The only specimens enumerated under the type which show any approach to zigzag branchlets, are Potanin's specimen from the same region and John R. Muir's specimens from western Szechuan. The specimens described as *S. sinobrahuica* var. *aridicola* may be considered as being nearest to f. *tortuosa*.

Spiraea siccanea (W. W. Sm.), spec. nov.

Spiraea yunnanensis Fr. var. *siccanea* W. W. Sm. in Not. Bot. Gard. Edinb. x. 69 (1917).

Frutex 1-1.5 m. altus ramis gracilibus teretibus, hornotinis adpresse villosulis, annotinis glabris purpureo-fuscis partim decorticantibus; gemmae ovoideae, pluriperulatae perulis ovatis glabris ciliolatis. Folia ramulorum floriferorum (turionum non vidi) obovata vel ovalia, rarius oblongo-ovalia, 0.8-2 cm. longa et 6-10 mm. lata, apice rotundata vel obtusa, mucronulata, basi late cuneata vel fere rotundata, supra medium inaequaliter dentata vel crenato-dentata dentibus mucronulatis, interdum indistincte trilobata, supra laete viridia et glabra, subtus glauca ad costam nervosque tantum laxe pilosa, basi 3- vel 5-nervia, ceterum utrinque nervis 1-2 instructa; petioli 1-2 mm. longi, villosuli. Flores albi, subumbellati, circiter 12-20 ramulos paucifoliatos pedunculo 5-10 mm. longo incluso 1-2 cm. longos terminantes; pedicelli 5-10 mm. longi ut pedunculus villosuli; calyx turbinatus, circ. 1 mm. longus, ut lobi triangulares acutiusculi subaequilongi laxe villosus; petala orbiculari-obovata, 3-4 mm. longa; stamina circ. 20, dimidia petala aequantia; discus conspicuus, annularis, 10-lobatus; carpidia villosula, stylo apicali fere 1 mm. longo coronata. Fructus non vidi.

CHINA. Y u n n a n : Lang-kong-Hoching mountains, Lat. 26° 16′

N., alt. 8000 ft., open dry situations, *G. Forrest,* no. 9912 (syntype of *S. yunnanensis* var. *siccanea*), May 1913 (shrub of 3-5 ft.; flowers white); Lang-kong-Hoching divide, Lat. 26° 10′ N., alt. 8000 ft., dry stony situations amongst scrub, *G. Forrest,* no. 9972 (syntype of *S. yunnanensis* var. *siccanea*) May 1913 (shrub of 3-5 ft.; flowers creamy white).

This seems to be distinct enough to be specifically separated from *S. yunnanensis* Franch. from which it is readily distinguished by the leaves being quite glabrous above and only loosely pilose on the veins beneath; no forms intermediate in pubescence were found among the numerous specimens seen of *S. yunnanensis,* except perhaps one specimen collected between Batang and Tachienlu by John R. Muir, in which the pubescence though rather slight, is present nevertheless on both sides; at the same time the specimen has very small, 4-8-flowered inflorescences, very small leaves and slightly tortuous branchlets.

Malus Rockii, sp. nov.

Arbor 8-10 m. alta, ramis pendulis; ramuli hornotini villosi, annotini glabrescentes fusci vel fusco-rubri; gemmae ovoideae, perulis atrofuscis ovatis medio villosis ceterum fere glabris. Folia chartacea, elliptica vel ovato-elliptica, ovata ad ovato-oblonga, 6-12 cm. longa et 3.5-7 cm. lata, acuminata, basi rotundata, rarius late cuneata, argute et adpresse inaequaliter serrulata, dentibus mucronato-acuminatis, supra costa sparse villosa excepta glabra et in costa et nervis glandulosa, impresso-reticulata, subtus pallida, in costa, nervis et venulis manifeste elevatis crispo-villosa, interdum sparse in facie villosula, venulis trabecularibus conspicuis; petioli 2-4 cm. longi, villosi. Flores non visi. Fructus 2-4 vel solitarii, pedicellis 2-4 cm. longis villosis suffulti, ovoidei vel subglobosi, basi in petiolum abrupte attenuata, apice juniores tandem plus minusve leviter attenuati 1-1.5 cm. longi, calyce tarde deciduo, juniores apice et basi villosuli lobis calycinis lanceolatis extus intusque villosis partim coronati, maturitate carminei, luciduli, 5-loculares.

CHINA. Yunnan: west of Talifu, Mekong watershed, en route to Young-chang and Tengyueh beyond Lampba, along watercourses, alt. 7000 ft., *J. F. Rock,* no. 6842 (type), Sept.-Oct. 1922 (tree with long drooping branches; fruits carmine, cherry-like); Litiping range, Mekong-Yangtze divide, east of Weihsi, *J. F. Rock,* no. 11552, Oct. 1923 (tree 25 ft.); Yangtze watershed, western slopes of Likiang Snow Range, *J. F. Rock,* no. 5346, May 30-June 6, 1922 (tree 35 ft.).

This new species is apparently nearest to *M. baccata* (L.) Borkh. but the fruits are larger, the calyx is tardily deciduous and the leaves are rather densely pubescent and reticulate beneath. From *M. pumila*

Mill. which it resembles somewhat in its leaves, it is farther removed by the deciduous calyx and the slender-stalked small fruit not impressed at the base and the apex; also the leaves are more strongly reticulate and rounded at base. One might compare *M. Rockii* with the hybrids between *M. baccata* and *M. pumila* or *M. prunifolia* (Willd.) Borkh., but the calyx seems to be always deciduous and the leaves are pubescent and reticulate beneath; moreover, hybrids between these two northern species cannot be expected to occur in Yunnan even as escapes from cultivation, and the three specimens cited above are apparently from spontaneous trees.

Malus hupehensis (Pamp.), comb. nov.

Pirus communis Pavolini in Nuov. Giorn. Bot. Ital. xv. 415 (1908).—Non Linnaeus.

Pirus hupehensis Pampanini in Nuov. Giorn. Bot. Ital. n. ser. xvii. 291 (1910).—Rehder in Sargent, Pl. Wilson. ii. 265, 300 (1915).

Pyrus baccata Hemsley in Jour. Linn. Soc. xxiii. 255 (1886), quoad plantam e Chekiang.—Diels in Bot. Jahrb. xxix. 387 (1900).—Non Linnaeus.

Pyrus spectabilis Hemsley in Jour. Linn. Soc. xxiii. 258 (1886), quoad plantam e Kiangsi et Hupeh.—Diels in Bot. Jahrb. xxix. 387 (1900).—Non Aiton.

Malus baccata var. *himalaica* Schneider, Ill. Handb. Laubholzk. i. 721, fig. 397s (1906), quoad plantam chinens. et fig.—Non Pyrus baccata var. himalaica Maxim.

Malus theifera Rehder in Sargent, Pl. Wilson. ii. 283 (1915); in Jour. Arnold Arb. v. 192 (1924); viii. 121 (1927); Man. Cult. Trees Shrubs, 395 (1927).—Chun, Chin. Econ. Trees, 173, fig. 65 (1922).—Hers in Jour. N. China Branch R. As. Soc. liii. 116 (1922); Liste Ess. Lign. Honan, 29 (1922).—Chung in Mem. Sci. Soc. China, i. 82 (Cat. Trees Shrubs China) (1924).—Hu & Chun, Icon. Pl. Sin. i. 32, t. (1927).—Wilson in Arnold Arb. Bull. ser. 3, i. 20, fig. (1927).

Pyrus theifera (Rehd.) Bailey in Rhodora, xviii. 155 (1916); Stand. Cycl. Hort. v. 2872 (1916).—Kew Handlist Trees Shrubs, ed. 3, p. 133 (1925).

It is rather unfortunate that the name of this species which as *Malus* or *Pyrus theifera* is already well known in horticultural literature as a highly ornamental Crabapple, has to be changed, but when examining last year in the Biondi herbarium at the Botanical Museum in Florence the type of Pampanini's *Pirus hupehensis,* I saw at once that this species is identical with my *Malus theifera.* When describing the latter species I had not seen a specimen of Pampanini's species, which according to the author's remarks was most closely related to *P. pashia* Buch.-Ham. and also to *P. communis* L. Owing to the world war, I was unable to obtain a specimen of the species from Florence and I, therefore,

mentioned (l. c. 265, 300) *P. hupehensis* among the doubtful species of *Pyrus*, but stated that it could not belong to *Pyrus* in the restricted sense, since the author described it as having three connate styles villous below. Later Pampanini apparently revised his opinion regarding the affinity of this species, since on a note dated December 1921 and pinned to the sheet of each type specimen he referred it to *Malus baccata* var. *himalaica* Schneid., making a new binomial combination of that variety under *Pirus* which, however, was never published. This identification, of course, came much closer to the true relationship of his *P. hupehensis*.

Malus hupehensis is widely distributed in mountainous regions of China at elevations of from 1000-2000 m. and extends south into Assam. It is represented in this herbarium by specimens from the following Chinese provinces: Shantung, Kiangsu, Honan, Chekiang, Kiangsi, Fukien, Hunan, Hupeh, Szechuan, Kweichou and Yunnan; also from Assam. The syntypes of *P. hupehensis* Pamp. were collected in northern Hupeh, Sian-men-kou (Silvestri, no. 939) and Ma-pau-scian (Silvestri, nos. 940, 9402); of nos. 939 and 940 there are photographs in this herbarium. The holotype of *M. theifera* also came from Hupeh, near Ichang (Wilson, no. 451), and the paratypes from other localities in Hupeh, from Shensi, Chekiang, Szechuan and Assam.

A form with rosy-pink flowers is the following:

Malus hupehensis f. **rosea** (Rehd.), comb. nov.

Malus theifera f. *rosea* Rehder in Sargent, Pl. Wilson. II. 284 (1915); Man. Cult. Trees Shrubs, 395 (1927).

Pyrus theifera var. *rosea* (Rehd.) Bailey in Rhodora, XVIII. 155 (1916); Stand. Cycl. Hort. v. 2872 (1916).

This form has been found in Hupeh; the type comes from Fang Hsien, (Wilson, no. 2980) and a paratype from Patung Hsien (Wilson, Veitch Exped. seed no. 766, Oct. 1900; specimen from Kew Bot. Gard., Wm. Bean, May 1914).

Prunus Slavinii Palmer, hybr. nov.

Prunus angustifolia var. *varians* Wight & Hedrick × *P. gracilis* Engelm. & Gray.

Frutex, 4-12 dm. altus, raro arborescens et ad 2-2.5 m. altus, dense ramosus, ramulis spinescentibus, novellis brunneo-rubescentibus glabris vel pubescentibus, vetustioribus cinereo-brunneis. Folia lanceolata vel ovato-lanceolata, tenuia sed firma, supra fere glabra, infra glabra vel pubescentia, venulis reticulatis; petioli graciles, 1-1.5 cm. longi glabri vel pubescentes, eglandulosi vel raro glandulosi. Flores 2-6-umbellati, pedicellis 9-12 mm. longis glabris vel pubescentibus. Fructus ovoideus vel subglobosus, 1.5-2.2 cm. longus, 1-2 cm. latus, ruber, pallide punctatus, vel rubro-luteus, succosus, esculentus.

Slender or arborescent shrubs .5 to 1 m. or rarely 2 to 2.5 m. tall, with numerous spreading or ascending somewhat spinescent branches, those of the last year's growth reddish-brown. Bark on old stems and branches dark gray or gray-brown, with pale lenticels. Leaves lanceolate or ovate-lanceolate 3.5-7 cm. long, 1-2.5 cm. wide, rounded or slightly subcordate at base, rounded, acute or short-acuminate at apex, finely serrate with shallow gland-tipped teeth, bluish-green and glabrous or sparsely pubescent above, paler and usually more densely pubescent beneath, sometimes only along the prominently reticulate veins, thin but firm in texture, on slender eglandular or rarely glandular petioles. Flowers appearing in March or early April before the leaves in 2-6-flowered umbels, on slender pubescent or nearly glabrous pedicels; ovary usually somewhat pubescent, rarely glabrous; calyx-teeth lanceolate, usually with entire margins, glabrous or slightly pubescent without, pubescent within; petals ovate, clawed, 3-4 mm. long; stamens numerous; anthers yellow or rarely red. Fruit ovoid or nearly globose, 1.5-2 cm. long, 1-2 cm. broad, bright to dark crimson with pale dots, or orange-yellow with red cheek; flesh yellow, becoming soft and succulent. Stone compressed-ovoid, 10-12 mm. long, 9-10 mm. wide, rounded at base, pointed at apex, slightly keeled and grooved on ventral side.

Growing in thickets, in sandy ground, within the range of the parent species and apparently always in close association with them. Range, from the Arkansas River valley in southeastern Kansas, through central Oklahoma, and probably to be expected also in eastern and central Texas.

North America. Kansas: Arkansas City, Cowley Co., *B. H. Slavin,* no. 164, April 10, July 4, 1914; *E. J. Palmer,* no. 21254, May 11, 1922. Oklahoma: Sapulpa, *B. H. Slavin,* no. 132, April 1, June 29, 1914; Muskogee, *B. H. Slavin,* no. 128, March 31, June 26, 1914; Oklahoma City, *B. H. Slavin,* no. 143, July 2, 1914, no. 144, April 4, July 2, 1914, no. 145, April 4, July 2, 1914, no. 146, April 4, July 2, 1914, no. 152, April 5, July 1, 1914 (**type**), no. 252, March 27, June 29, 1916; Norman, *B. H. Slavin,* no. 251, March 27, June 29, 1916; Chickasaw, *B. H. Slavin,* no. 257, March 29, 1916, no. 259, March 29, June 30, 1916, no. 260, June 30, 1916, no. 262, March 29, June 30, 1916; Kingfisher, *B. H. Slavin,* no. 329, April 12, July 5, 1916, no. 330, April 12, July 5, 1916, no. 331, April 12, July 5, 1916, no. 332, April 12, July 5, 1916, no. 334, July 5, 1916; Anadarko, *E. J. Palmer,* no. 12601, July 20, 1917. Also cultivated in the Arnold Arboretum and in Durand-Eastman Park, Rochester, N. Y.

The Chickasaw Plum (*Prunus angustifolia* Marsh.) is widely dis-

tributed in the southern states, from Maryland and Delaware to Florida, Oklahoma, and Texas. In the western part of its range, the var. *varians* Wight & Hedrick, is the commoner form, and it is found abundantly in sandy soil in central Kansas, Oklahoma, and northwestern, central and eastern Texas, where it often forms large thickets of spiny shrubs, 4 to 6 or eight feet in height. The yellow or red fruit matures early and is often of excellent quality. The leaves are prevailingly lanceolate, thin, nearly or quite glabrous, and with only the mid-veins prominent. They are usually conduplicate, making them appear narrower than they really are.

The Sand Plum (*Prunus gracilis* Engelm. & Gray) is found in the western part of the same range, from the valley of the Arkansas River, in southeastern Kansas, and along the western border of southern Arkansas, through most of Oklahoma and eastern Texas as far west as the Brazos River. It is a low slender shrub, usually from 1 to 4 feet in height. The leaves are oval or ovate, gray-green, of firm texture, slightly pubescent above and densely so beneath, and with prominent reticulate veins. The fruit is slightly smaller than that of the Chickasaw Plum, and is edible. It is sometimes borne in such profusion as to weigh the slender branches to the ground. The two species bloom simultaneously and apparently hybridize freely, judging by the number of specimens found.

Prunus Slavinii is quite intermediate in habit and character between the two parent species, and different individuals differ considerably in size and in the pubescence and prominence of the reticulation of the leaves. The specific name is for Mr. B. H. Slavin, superintendent of the splendid park system of Rochester, N. Y., who first collected this interesting Plum and brought it into cultivation there and at the Arnold Arboretum. The type specimen and all of the other numbers cited here are in the herbarium of the Arnold Arboretum.

ERNEST J. PALMER

Calophaca sinica, sp. nov.

Frutex erectus ramis robustis, hornotinis dense albido-pubescentibus, annotinis cortice purpureo-fusco laminis soluto ochraceo-albidis. Stipulae scariosae diu persistentes. Folia pinnata, cum petiolo 3-5 cm. longa, pleraque 7-foliolata; foliola chartacea, ovalia vel obovato-ovalia, 12-18 mm. longa et 7-12 mm. lata, apice rotundata vel truncata, basi rotundata et saepe leviter subcordata, supra cinereo-viridia, maturitate fere glabra, subtus pallidiora, minute et laxe villosula, reticulo venularum satis manifesto, nervis utrinsecus 5-6, supra leviter subtus magis elevatis; petioluli villosi, 1 mm. breviores; petioli 5-12 mm. longi, ut rhachis albido-villosuli. Pedunculi circiter 4 cm. longi longe patentim

villosi et apicem versus stipitato-glandulosi, pauciflori; flores non visi; legumen oblongum, circ. 3 cm. longum, villosum, glandulis longe stipitatis crebris munitum, calyce villoso et stipitato-glanduloso lobis lineari-lanceolatis tubum subaequantibus suffultum.

China. Shansi: Chiao-cheng hsien, alt. 3000 ft., *W. Y. Hsia*, no. 1032, May 13, 1929.

The discovery of this new species in Shansi is of interest particularly for the reason that it extends the range of the genus farther to the East into the flora of China.

The genus in its restricted sense (excl. of sect. *Chesneya*) ranges from southern Russia and the Caspian region through Turkestan (*C. wolgarica* Fisch.), Bokhara (*C. grandiflora* Reg.) Tian-shan (*C. wolgarica* var. *tianschanica* [B. Fedtch.] Popov) to Dsungaria (*C. Hovenii* Schrenk) and now extends into northeastern China.

From *C. wolgarica* the new species differs chiefly in the fewer and larger leaflets, the absence of glands from the rhachis and the lower half of the peduncle; from *C. grandiflora* in the fewer leaflets, few-flowered raceme and the stalked glands of the legume and from *C. Hovenii* chiefly in the larger leaflets the spreading pubescence and the presence of stipitate glands on the inflorescence and the legume.

Acer sect. **Macrantha** Pax, emend. Rehd.

The section Macrantha is one of the most difficult of the sections of *Acer* on account of the rather uniform character of the inflorescence, flowers and fruit and of the variability of the foliage with apparently intermediate forms between the species. The section is here limited as proposed by me in 1911 (in Sargent, Pl. Wilson. I. 92) where I included into this section proposed by Pax in 1886 (in Bot. Jahrb. VII. 244) several species referred by Pax to his sect. Indivisa, namely *A. sikkimense* Miq., *A. Hookeri* Miq., *A. Davidi* Franch., *A. laxiflorum* Pax and *A. crataegifolium* Sieb. & Zucc., but I excluded *A. parviflorum* Franch. & Sav. and *A. erosum* Pax which is a synonym of *A. caudatum* Wall. var. *multiserratum* (Maxim.) Rehd. and belongs like *A. parviflorum* to the sect. Spicata. Another species erroneously referred to the Macrantha by Handel-Mazzetti and by Fang, is *A. Wardii* W. W. Sm. (*A. mirabile* Hand.-Mazz.) which belongs to the sect. Spicata into the affinity of *A. sinense* Pax; the inflorescence, though typically paniculate, is sometimes reduced to a simple raceme as in the type of *A. mirabile*, but in the bracted and opposite rather long and ascending pedicels and in the flowers it differs from the Macrantha section. Besides the seven Chinese species distinguished here, there are eight species in Eastern Asia outside of China (*A. crataegifolium* Maxim.,

A. capillipes Maxim., *A. tegmentosum* Maxim., *A. rufinerve* S. & Z., *A. micranthum* S. & Z., *A. Tschonoskii* Maxim., *A. morrisonense* Hay. and *A. rubescens* Hay.), three in India (*A. sikkimense* Miq., *A. Hookeri* Miq. and *A. pectinatum* Wall.) and one in Eastern North America (*A. pennsylvanicum* L.).

Of the Chinese species *A. Davidi* is the most widely distributed and is found in all provinces of China except in Hopei and Shantung. The other species are of more restricted distribution and each seems to occupy a fairly well defined area. *Acer laxiflorum* Pax is restricted to Szechuan and southeastern Tibet. *Acer taronense* ranges from northern Szechuan through western Yunnan to eastern Tibet and northern Burma. *Acer Forrestii* is found in southwestern Szechuan and western Yunnan. *Acer Maximowiczii* is a northwestern species and extends from southern Shensi and Kansu into northern Szechuan and into Hupeh; a rather distinct form is found in Kweichou. *Acer Grosseri* with var. *Hersii* is found in northern China from Kansu to Hopei and extends south to Hupeh and through Honan to Anhwei. *Acer Metcalfii* is known so far only from Kwangtung and from Hunan, if *A. Davidi* f. *trilobata* is identical with it.

As neither inflorescence, nor flowers or fruits in this section seems to show distinctive and reliable characters, the following key is based exclusively on the leaves which allows identification of both flowering and fruiting specimens.

KEY TO THE CHINESE SPECIES

A. Leaves not lobed, doubly crenate-serrate with obtusish or acutish teeth ..... 1. *A. Davidi*

AA. Leaves more or less lobed.

B. Margin of leaves with 5 or more acute or acuminate teeth to 1 cm.

C. Leaves with rusty pubescence on the veins beneath;

D. Lateral lobes short, acute; flowers red (?always). 2. *A. laxiflorum*

DD. Lateral lobes elongated, acuminate; teeth acuminate to aristate. ..... 3. *A. taronense*

CC. Leaves glabrous beneath except axillary beards in some species.

D. Leaves doubly and sharply serrate with acuminate teeth; middle lobe elongated, the lateral below the middle of the leaf, long acuminate, sometimes short on part of the leaves.

E. Leaves 6-12 cm. long without or occasionally with small basal lobes, finely and closely doubly serrulate; lateral lobes sometimes short and acute, pointing forward. 4. *A. Forrestii*

EE. Leaves 4-8 cm. long, with distinct basal lobes rarely without, incisely doubly serrate or lobulate; lateral lobes always long-acuminate, spreading...5. *A. Maximowiczii*

DD. Leaves unequally or doubly serrate with obtusish mucronulate teeth; middle lobe triangular-ovate; lateral lobes short, acute or short acuminate, rarely long-acuminate. .....6. *A. Grosseri*

BB. Margins of leaves coarsely and irregularly dentate with 2-3, rarely 4, obtuse teeth to 1 cm., lobes acuminate with entire acumen. .....7. *A. Metcalfii*

1. **Acer Davidi** Franchet in Nouv. Arch. Mus. Paris, sér. 2 VIII. 212 (Pl. David. II. 30) (1884).—Pax in Engler, Pflanzenr. IV.-163, p. 36 (1902).—Rehder in Sargent, Trees & Shrubs, I. 167, t. 83 (1905); in Jour. Arnold Arb. VII. 221 (1926); VIII. 163 (1927); IX. 90 (1928).—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 174 (1932).—Fig. 1.

Acer Davidi var. *glabrescens* Pax in Hooker, Icon. XIX. sub t. 1897 (1889); in Bot. Jahrb. XXIX. 449 (1900); in Engler, Pflanzenr. IV.-163, p. 36 (1902).—Rehder in Sargent, Trees and Shrubs, I. 167 (1905).

Acer Davidi l. *tomentellum* Schwerin in Gartenfl. XLII. 230 (1893).—Pax in Engler, Pflanzenr. IV.-163, p. 36 (1902) "var. α."

Acer Davidi var. *horizontale* Rehder in Sargent, Trees and Shrubs, I. 168 (1905), pro parte, quoad specim. Wilson, no. 1882.—Non Pax.

Acer Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. X. 432 (1911).

Acer laxiflorum var. *integrifolium* Fang in Contrib. Biol. Lab. Sci. Soc. China, VII. 174 (1932).

DISTRIBUTION: Kansu, Kiangsi, Chekiang, Anhwei, Kiangsi, Hunan, Kwangtung, Kwangsi, Kweichou, Szechuan, Yunnan and southeastern Tibet.

I have seen numerous specimens from all the provinces of China named above. This shows that the species is widely distributed throughout China except the northern and northeastern provinces Shensi, Shansi, Hopei, Honan and Shantung.

I do not think that var. *glabrescens* Pax is distinct enough to be separated as a variety or form. Its holotype, Henry, no. 7085, shows even on the mature leaves remnants of the brown tomentum. Var. *tomentellum* Schwerin represents the typical form.

Acer Cavaleriei Lévl. of which I have a duplicate of the holotype, Cavalerie, no. 3345, before me differs slightly in the rather narrow oblong leaves 2.8-3.2 cm. wide, rounded, not subcordate at base, quite glabrous beneath, with simple and rather slight crenate serration and an entire acumen; the wings of the fruit spreading horizontally.

Acer laxiflorum var. *integrifolium* Fang from Mt. Omei, Szechuan, of which I have a duplicate of the holotype, Fang, no. 2692, does not seem to differ from *A. Davidi* except that the leaves are comparatively small.

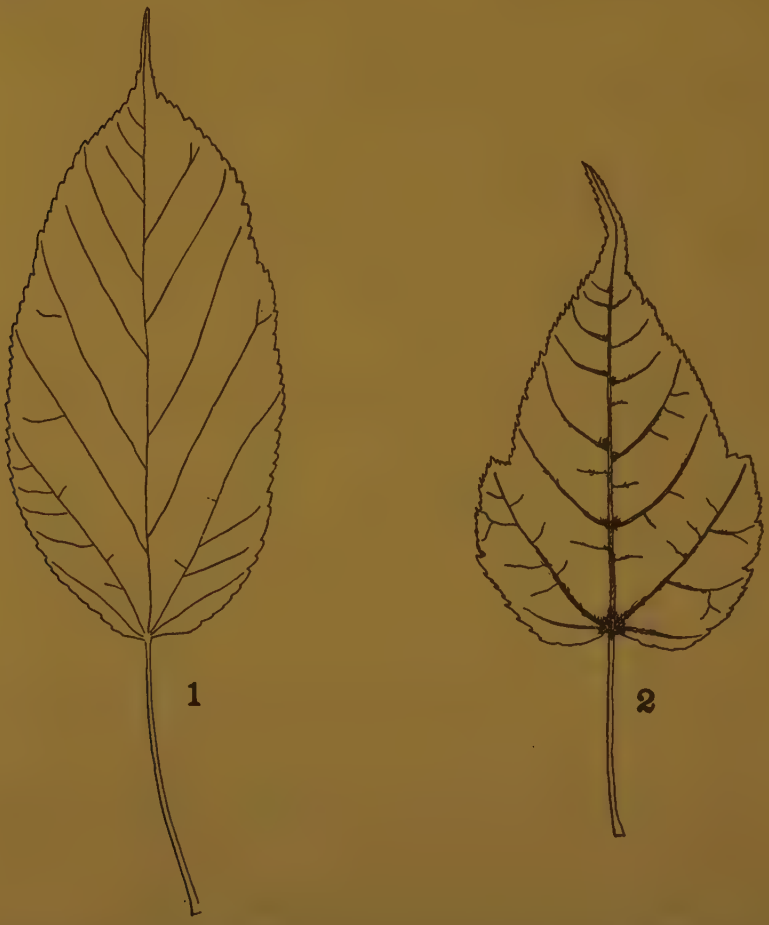


FIG. 1. ACER DAVIDI Franch.: leaf (2/3 nat. size) from Wilson, no. 1005a Mupin (type locality).—FIG. 2. ACER LAXIFLORUM Pax: leaf (2/3 nat. size) from Faber, no. 433, Mt. Omei (syntype).

2. **Acer laxiflorum** Pax in Engler, Pflanzenr. IV.-163, p. 36 (Acer.) (1902).—Rehder in Sargent, Trees & Shrubs, I. 180 (1905); in Sargent, Pl. Wilson. I. 93 (1911), excl. synon.—Fang in Contrib. Biol. Lab. Sci. Soc. China, VII. 178 (1932).—Fig. 2.

SZECHUAN: Mt. Omei, *E. Faber,* no. 453 (syntype), *E. H. Wilson,* Veitch Exp. 3349a, *W. P. Fang,* no. 2874; Nanchuan Hsien, *W. P. Fang,* no. 1191; Pan-han-shan, *E. H. Wilson,* nos. 1904, 4142; Kuan hsien, *W. P. Fang,* no. 2369; Wenchuan-Hsien, *E. H. Wilson,* nos. 1309, 4099, 4108; Wa-shan, *E. H. Wilson,* no. 1154; Mupin, *E. H. Wilson,* nos. 1007, 1007a, 1069, 1234; Tachienlu, *E. H. Wilson,* no. 1309, *W. P. Fang,* no. 3664. SOUTHEASTERN TIBET: Tsarong, *G. Forrest,* no. 21671; Mt. Kenyichunpo and region of Champutong, Salween-Irrawadi watershed, *J. F. Rock,* no. 10242.

Acer laxiflorum is closely related to *A. Davidi* Fr. from which it may be distinguished by the lobed leaves with sharper acute serration, longer and slenderer acumen and with pubescent veins beneath. It also is very close to *A. Forrestii* W. W. Sm. from which it differs in its broader and larger leaves pubescent on the veins beneath though the pubescence is sometimes rather slight. The flowers and young fruits of *A. laxiflorum* are more or less purple or red, but occasionally the flowers may be greenish as in Wilson's no. 1309, though the fruits of the same number are reddish. The flowers of the two other species are always greenish.

Acer laxiflorum var. **longiphyllum** Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 179 (1932).

This variety based on Fang's no. 4513 from Ma-pien-hsien, which I have not seen, does not appear according to the description to differ much, if at all, from the type. Neither have I seen *A. laxiflorum* var. *ningpoense* Pax in Engler, Pflanzenr. IV.-163, p. 36 (1902).

3. **Acer taronense** Handel-Mazzetti in Anz. Akad. Wiss. Wien, 1924, p. 84 (Pl. Nov. Sin. Forts. 25, p. 3).—Fig. 3.

Acer laxiflorum Pax var. *longilobum* Rehder in Sargent, Pl. Wilson. I. 94 (1911), excl. specim. Wilson, 4108.

SZECHUAN: Chin-ting-shan, *E. H. Wilson,* no. 1927 (type of *A. laxiflorum* var. *longilobum*); Tu-ti-liang Mts., Lungan-fu, *E. H. Wilson,* no. 4509. YUNNAN: "prope fines tibeto-birmanicas inter fluvios Ludjiang (Salween) et Djiou-djiang (Irrawaddi), *Handel-Mazzetti,* no. 9385 (type of *A. taronense*); Mt. Gitsa west of Mekong and north of Wei-hsi, *J. F. Rock,* no. 18425; without precise locality, *G. Forrest,* nos. 8990 and 9059. EASTERN TIBET: without precise locality, *G. Forrest,* nos. 26317 and 26581. UPPER BURMA: *G. Forrest,* 26501 and 27269.

Acer taronense is closely related to *A. laxiflorum* Pax, but the leaves differ in the caudate-acuminate lateral lobes, broader and comparatively shorter middle lobe, finer and closer serration with aristate teeth and more densely pubescent veins beneath, though glabrescent at maturity. In the less fine and close serration the Szechuan specimens approach *A. laxiflorum,* but the lateral lobes are caudate-acuminate. On none

of the specimens is the pubescence quite as dense on the veins as in the type specimen. The racemes of the Burma and Tibetan specimens are quite long (about 7-8 cm.) and many-flowered, while in Wilson's no. 1927 they are only 4-5 cm. long and about 10-flowered. It is also closely related to *A. pectinatum* Wall., which is distinguished by the usually 5-lobed leaves more deeply cordate at base and glabrous beneath except the bearded axils; in its very close aristate serration it approaches the specimens of *A. taronense* from Burma.

FIG. 3. ACER TARONENSE Hand.-Mazz.: leaf (2/3 nat. size) from Handel-Mazzetti, no. 9385 (holotype).—FIG. 4. ACER FORRESTII Diels: leaf (2/3 nat. size) from G. Forrest, no. 2106 (holotype).

4. **Acer Forrestii** Diels in Not. Bot. Gard. Edinb. v. 165 (1912).—Fig. 4.

Acer laxiflorum Rehder in Sargent, Pl. Wilson. I. 93 (1911), in part.—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 178 (1932), in part.—Non Pax.

SOUTHERN SZECHUAN: east of Ning-yuan-fu, *C. Schneider,* no. 959; between Ouentin and Kalapa, *C. Schneider,* no. 1462; between Hunke and Woloho, *C. Schneider,* no. 1499; Kingdom of Muli, *J. F. Rock,* nos.

17993, 18046 and 18230, *G. Forrest,* no. 21337. YUNNAN: Likiang range, *G. Forrest,* no. 2106 (holotype), *C. Schneider,* nos. 1909, 3281, 3338, 3338a, *J. F. Rock,* nos. 3490, 3761, 4231, 5105, 5404; north of Wei-hsi, *J. F. Rock,* no. 17062; near Pe-yen-tsin, *S. Ten,* no. 548; between Chien-chuan plain and Mekong drainage basin, *J. F. Rock,* no. 8630; Chien-chuan-Mekong divide, *G. Forrest,* no. 22380; Mekong-Salween divide, *G. Forrest,* no. 20009, *J. F. Rock,* no. 8893; without precise locality, *G. Forrest,* nos. 10063, 11226, 11279.

Acer Forrestii is closely related to *A. laxiflorum* Pax, but differs in the glabrous and glaucescent under-side of its leaves; the forms with longer acuminate lobes approach *A. Maximowiczii* Pax, but can be distinguished by the finer and closer serration, the absence of the basal pair of lobes and the glaucescent under-side. The flowers are mostly greenish, but Rock's no. 8893 has red flowers; also the fruits of Rock's no. 5404, of Schneider's no. 1909 and Forrest's no. 20009 are distinctly red or reddish.

Acer Forrestii f. **caudatilobum,** forma nova.—Fig. 5.

A typo differt lobis longe caudato-acuminatis, lobis lateralibus lobo medio plerumque plus quam dimidio longioribus.

YUNNAN: Yangtze watershed, western slopes of Likiang Snow Range, *J. F. Rock,* no. 4149, May 30—June 6, 1922 (tree 10 m.; petioles and stems red).

With its caudate-acuminate lobes, the lateral ones mostly more than half as long as the middle lobe, this form looks so strikingly different from the type that it seems desirable to distinguish it as a form.

5. **Acer Maximowiczii** Pax in Hooker, Icon. XIX. sub t. 1897 (1889); in Engler, Pflanzenr. IV.-163, p. 70 (1902).—Rehder in Jour. Arnold Arb. VII. 223 (1926); IX. 90 (1928).—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 180 (1932).—Fig. 6.

Acer urophyllum Maximowicz in Act. Hort. Petrop. XI. 105 (1890).—Rehder in Sargent, Trees and Shrubs, I. 169, t. 84 (1905).

HUPEH: without precise locality, *A. Henry,* nos. 6857 (syntype) and 6783, *E. H. Wilson,* Veitch Exp. nos. 1891 and 2343; Mt. Ngo-san, *Hugh Scallan;* Chang-yang, *E. H. Wilson,* Veitch Exp. no. 724; South Wushan, *E. H. Wilson,* no. 229; Fang-hsien, *E. H. Wilson,* nos. 355 (in part), 1914 (in part) and 4427; Hsing-shan-hsien, *E. H. Wilson,* nos. 355 (in part) and 1914 (in part); Ichang, *E. H. Wilson,* nos. 355 (in part) and 1914 (in part); Hsao-lung-T'an, *W. Y. Chun,* nos. 4220 and 4618; Shin-tien-tze, *W. Y. Chun,* 4030. SHENSI: Tai-pei-shan, *W. Purdom,* nos. 947 and 948. KANSU: Lower Tebbu country, Want-sang forests, *J. F. Rock,* nos. 14682, 14703, 14706, 14730, 14814, 14855,

15031, 15041 and 15047; Tsaushi-ku, *J. F. Rock,* no. 14735; Dayaya, *J. F. Rock,* no. 14784; Pezhu valley, *J. F. Rock,* no. 14946; Tsaoshiku, *J. F. Rock,* no. 14998; Lien-hoa-shan, Shanshen-miao, *J. F. Rock,* no. 13488; vicinity of Choni, *R. C. Ching,* no. 1009. SZECHUAN: Sing-pan-hsien, *E. H. Wilson,* nos. 4100 and 4513, *W. P. Fang,* no. 4171; Nanchuan-hsien, *W. P. Fang,* no. 931. KWEICHOU: Fan-ching-shan, *Steward, Chiao & Cheo,* no. 500.

FIG. 5. ACER FORRESTII f. CAUDATILOBUM Rehd.: leaf (2/3 nat. size) from J. F. Rock, no. 4149 (holotype).

Acer Maximowiczii is very similar to *A. Forrestii* Diels, but may be distinguished by the smaller leaves with coarser, distinctly double and even lobulate serration, by the presence of two small basal lobes, though often much reduced or sometimes lacking, and by the more elongated and spreading lateral lobes. In the Kansu specimens collected by Rock the basal lobe is mostly lacking, but the serration and the general

shape and size of the leaf is that of *A. Maximowiczii*. The specimen from Kweichou (Steward, no. 500) has much larger leaves up to 10.5 cm. long and 8.5 cm. wide with the teeth more acuminate, but they are distinctly 5-lobed and lobulate and the lobes abruptly contracted in the long acumen. Possibly it should be considered a distinct variety.

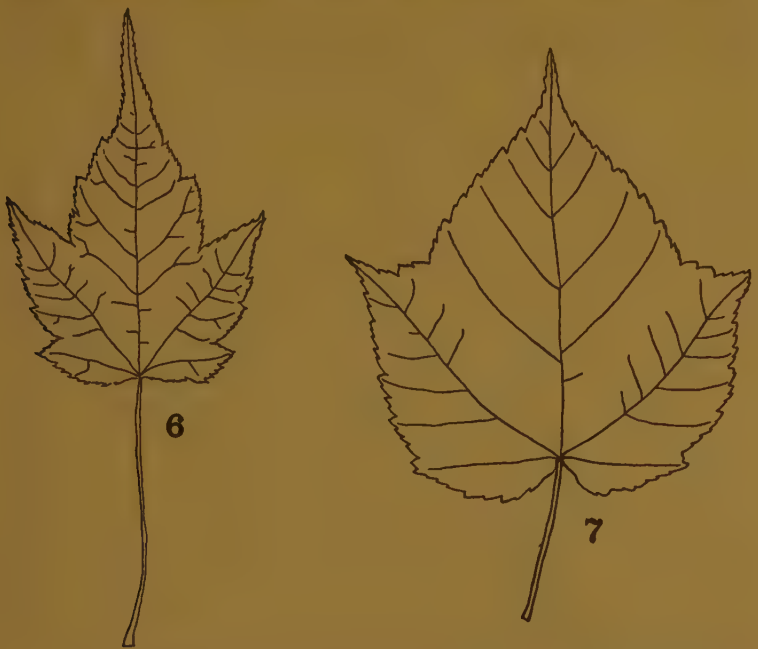


FIG. 6. ACER MAXIMOWICZII Pax: leaf (2/3 nat. size) from Henry, no. 6783. FIG. 7. ACER GROSSERI Pax: leaf (2/3 nat. size) from Harry Smith, no. 7932, Shansi.

Acer Grosseri Pax in Engler, Pflanzenr. IV.-163, p. 80 (1902).—Rehder in Sargent, Trees & Shrubs, I. 181 (1905); in Jour. Arnold Arb. VII. 222 (1926); VIII. 163 (1927); IX. 90 (1928).—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 181 (1932).—Fig. 7.

Acer Davidii var. γ *horizontalis* Pax in Engler, Pflanzenr. IV.-163, p. 79 (1902); in Bot. Jahrb. XXXVI. beibl. LXXXII. 72 (1905).—Rehder in Sargent, Trees and Shrubs, I. 168 (1905), excl. Wilson. no. 1882.—Hers in Jour. N. China Branch R. As. Soc. LIII. 106 (1922); Liste Ess. Lign. Honan Sept. 1 (1922).

Acer Davidii var. *glabrescens* Pax in Bot. Jahrb. XXXVI. beibl. LXXXII. 73 (1905).—Non Pax (1889).

Acer Pavolinii Pampanini in Nouv. Giorn. Bot. Ital. XVII. 422 (1910).

Acer Hersii Rehder in Jour. Arnold Arb. III. 217 (1922), pro parte.

HOPEI: without precise locality, *Père Chanet*, no. 90. SOUTHERN SHANSI: Chich-hsin, *Harry Smith*, no. 7932, 5895; Shih-li-p'o-shan, *Harry Smith*, no. 6780; Mien-shan, Lin-shih-hsien, *T. Tang*, no. 970; Chin-yuan, Lin-kon-shan, *K. Ling*, no. 9346. SHENSI: Kan-y-san, *G. Giraldi*, no. 2121 (holotype; photo. in A. A.); Lin-tou-san, *G. Giraldi*, July 14, 1897; Mt. Marg-hua-san, west of Singan-fu, *G. Giraldi*, Oct.-Nov. 1894; Tai-pei-shan, *W. Purdom*, no. 949; Thui-kio-tsuen and Mt. Ngo-san, *Hugh Scallan*, in 1899; Hua-shan, *J. Hers*, no. 3080; 60 km. south of Sian-fu, *J. Hers*, nos. 2950, 2999; Lung-chow, *J. Hers*, no. 2359. KANSU: Lower Tebbu country, Mayaku, *J. F. Rock*, no. 15053. HUPEH: Ku-tcen, *C. Silvestri*, no. 1377 (syntype of *A. Pavolinii;* photo. in A. A.); Ou-tan-scian, *C. Silvestri*, nos. 1370, 1371. NORTH HONAN: Sung-shien, *J. Hers*, no. 533; Lu-shi, *J. Hers*, no. 1169. ANHWEI: Chu-hwa-shan, *R. C. Ching*, no. 2613.

Acer Grosseri is very close to *A. Davidi* from which it differs chiefly in its lobed leaves and the somewhat sharper serration, but the lobes sometimes are very short or nearly obsolete which makes it difficult to separate the two species. Such intermediate specimens are e. g. K. Ling, no. 9346, from Shensi, and Giraldi's Mt. Mong-hua-san specimen and Purdom no. 949 from Shensi, but the slightly lobulate margin, the sharper serration and the glabrous under side refer them to *A. Grosseri*.

In the type specimen the middle lobe of the leaf is broadly triangular-ovate, while in other specimens it becomes elongated and oblong-ovate which gives the leaves a resemblance to those of *A. laxiflorum* Pax, but the latter has the leaves pubescent on the veins of the under side and a sharper and deeper serration.

Acer Grosseri var. **Hersii** (Rehd.), comb. nov.—Fig. 8.

Acer Hersii Rehder in Jour. Arnold Arb. III. 217 (1922); VII. 222 (1926); VIII. 163 (1927).—Fang in Contrib. Biol. Lab. Soc. Sci. China, VII. 180 (1932).

Acer sp. allied to *A. Grosseri* Bailey, Gent. Herb. I. 35 (1920).

HONAN: Teng-feng-hsien, *J. Hers*, nos. 219 (holotype) and 2780; Tsi-yuan-hsien, *J. Hers*, nos. 1739 and 2800; Kikung-shan, *A. N. Steward*, no. 9768 (in part) Aug. 3, 1925. HUPEH: Kikungshan, *A. N. Steward*, no. 9768 (in part) July 1925; *L. H. Bailey*, June 16, 1917. ANHWEI: Tsin-tai, Chu-kwa-shan, *R. C. Ching*, no. 2789.

This variety differs from the type in the more elongated long-acuminate lateral lobes, most pronounced in Steward's no. 9768 from the Kikung-shan, in which the lateral lobes are nearly as long as the middle lobe.

Acer Metcalfii, sp. nov.—Fig. 9.

Acer Davidi forma *trilobata* Diels in Notizbl. Bot. Gard. Mus. Berlin, XI. 211 (1931).—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 177 (1932).

Arbor 10-metralis, glaberrima, ramulis laevibus fuscis vel flavescentibus. Folia decidua, subcoriacea, trilobata, basi subcordata, lobis medio et lateralibus caudato-acuminatis acumine basi except integro, grosse inaequaliter serrato-dentatis dentibus obtusiusculis, nervis lateralibus

FIG. 8. ACER GROSSERI var. HERSII Rehd.: leaf (2/3 nat. size) from A. N. Steward, no. 9768, Honan.

lobi medii 8-9 in dentes exeuntibus et tantum dentibus duobus vel uno vel nullo inter nervos laterales, utrinque in sicco conspicue reticulata et brunneo-viridia subtus vix pallidiora; petioli circiter 2.5 cm. longi. Flores non visi. Racemi fructiferi 5-6 cm. longi, fructibus 4-6, nuculis compressis nervosis fere horizontalibus circ. 6 mm. longis, alis leviter ascendentibus angulum latum formantibus, nuculo excluso 1.5-2 cm. longis et 6-7 mm. latis.

KWANGTUNG: Lung-tan Mountain, near Iu, Herb. Canton Christ.

Coll. no. 12135, May 22-July 5, 1924 (type). SOUTHERN HUNAN: without precise locality, *S. S. Sin,* no. 298, May to Aug. 1926.

This new species is closely related to *A. Grosseri* var. *Hersii* Rehd. but is easily distinguished by the subcoriaceous leaves reticulate on both sides remotely and coarsely dentate-serrate with obtusish teeth; between the teeth terminating the lateral veins, there are only one or two or rarely three secondary teeth and none at all toward the apex of the lobes. Of *Acer Davidi* f. *trilobata* I have before me only a photograph

FIG. 9. ACER METCALFII Rehd.: leaf (2/3 nat. size) from Canton Christ. Coll. no. 12135 (holotype).

of the type which shows a serration somewhat less coarse and remote than that of the type of *A. Metcalfii,* but it certainly is referable rather to that species than to *A. Grosseri* var. *Hersii.*

I take pleasure in naming this new species in honor of Dr. F. P. Metcalf, who recognized the specimen as new and marked it thus in this herbarium; his recent paper on the species of the section Integrifolia (Lingnan Sci. Jour. XI. 193-210. 1932) is a valuable contribution to the knowledge of the genus *Acer.*

NOTES ON THE LIGNEOUS PLANTS DESCRIBED BY LEVEILLE FROM EASTERN ASIA[1]

Alfred Rehder

RUTACEAE

Zanthoxylum simulans Hance in Ann. Sci. Nat. Bot. sér. 5, v. 208 (1866), *"Xanthoxylum."*—Rehder in Jour. Arnold Arb. vii. 181 (1826).

Zanthoxylum Bungei Planchon in Ann. Sci. Nat. Bot. sér. 3, xix. 82 (1853), nomen.—Hance in Jour. Bot. xiii. 131 (1875) *"Xanthoxylum";* non Ann. Sci. Nat. sér. v. 209 (1866).

Zanthoxylum Argyi Léveillé in Mem. Acad. Ci. Barcelona, xii. 560 (Cat. Pl. Kiang-Sou, 20) (1916).—**Synon. nov.**

China. K i a n g s u : montagnes, *d'Argy,* May (1846-66) (holotype of *Z. Argyi;* merotype in A. A.).

Zanthoxylum stenophyllum Hemsley in Ann. Bot. ix. 147 (1895).—Rehder & Wilson in Sargent, Pl. Wilson. ii. 127 (1914).

Zanthoxylum Esquirolii Léveillé in Fedde, Rep. Spec. Nov. xiii. 266 (1914); Fl. Kouy-Tchéou, 377 (1915).—**Synon. nov.**

China. K w e i c h o u : without locality, *J. Esquirol,* no. 425, June 1904 "arbrisseau" (holotype of *Z. Esquirolii;* photo. and fragments in A. A.).

Zanthoxylum Chaffanjoni Léveillé in Fedde, Rep. Spec. Nov. xiii. 266 (1914).

Zanthoxylum oxyphyllum Léveillé, Fl. Kouy-Tchéou, 377 (1915).—Non Edgeworth.

China. K w e i c h o u : environs de Kouy-yang, mont du Collège, *J. Chaffonjon* in herb. Bodinier, no. 2171, April 12, 1898 "arbuste liane épineux" (holotype; photo. in A. A.).

This species which belongs to the section Fagara D. Don differs from *Z. oxyphyllum* Edgew. to which it was referred by Léveillé in 1916 (l. c.) chiefly in the 1-3 pairs of minutely serrulate leaflets, in the small inflorescences and the small flowers with the perianth only about 3 mm. long. It is rather similar to *Z. cuspidatum* Champ. but easily distinguished by the serrulate fewer leaflets, the smaller inflorescence and the distinct pedicels 1-3 mm. long; from *Z. nitidum* DC. it differs in the acuminate serrulate leaflets.

[1]Continued from vol. xiii. 332; for preceding parts see vol. x. 108-132, 184-196 and vol. xii. 275-281.

Zanthoxylum dissitum Hemsley in Jour. Linn. Soc. XXIII. 106 (1886).—Rehder & Wilson in Sargent, Pl. Wilson. II. 128 (1914).—Léveillé, Fl. Kouy-Tchéou, 377 (1915).

Zanthoxylum Bodinieri Léveillé in Fedde Rep. Spec. Nov. XIII. 266 (1914).

CHINA. K w e i c h o u : environs de Kouy-yang, mont du Collège, trou au pied de la montagne de Ste. Anne (item à Tsin-gay, Che-téou-tchay) *E. Bodinier,* no. 2058 (in part; flower buds), Feb. 10, 1898 "grande liane épineuse," (syntype of *Z. Bodinieri*); environs de Gan-pin, torrent des Ligularia, *L. Martin* & *E. Bodinier,* no. 2058 (in part; open flowers), Feb. 25, 1898 "grande liane épineuse" (syntype of *Z. Bodinieri;* photo in A. A.); Pin-fa, bois, *J. Cavalerie,* no. 748, Dec. 4, 1902 "fruit à odeur forte" (syntype of *Z. Bodinieri;* photo. in A. A.).

Zanthoxylum Bodinieri was enumerated by Léveillé in 1915 as a synonym of *Z. dissitum* Hemsl.

Zanthoxylum odoratum Léveillé in Fedde, Rep. Spec. Nov. XIII. 266 (1914).

Evodia odorata Léveillé in Fedde, Rep. Spec. Nov. IX. 458 (1911); Fl. Kouy-Tchéou, 375 (key) (1915).

Fagara gigantea Handel-Mazzetti in Akad. Anz. Wien, 1921, p. 64 (Pl. Nov. Sin. Forts. 10, p. 2) (1921).—**Synon. nov.**

Zanthoxylum giganteum (Hand.-Mazz.) Rehder in Jour. Arnold Arb. VIII. 151 (1927).

CHINA. K w e i c h o u : Ma-jo, *J. Cavalerie,* no. 2978, Aug. 1908 "odeur forte et agreable" (holotype of *Evodia odorata,* in fruit; photo. in A. A.); Pin-fa, montagne *J. Cavalerie,* no. 1771, April 17, 1904, "trouvé un pied seulement, 3 ou 4 m. de haut; les nombreuses fleurs blan. avaient une odeur forte et agreable"; cited under *Zanthoxylum odoratum;* photo. in A. A.); Ta-hi-yen, Feng-hsiang-ping, on bushy slope, alt. 1700 m., *Steward, Chiao* & *Cheo,* no. 708, Oct. 18, 1931 (tree 8 m. high, 40 cm. diam.; fruit reddish). H u n a n : in monte Yunschan prope urbem Wukang, in silva, alt. 1150-1250 m., *Handel-Mazzetti,* no. 12327, Aug. 8, 1918 "arbor 15 m.; fl. virenti-flavi cum foliis citriodori" (holotype of *Fagara gigantea;* isotype in A. A.).

Under *Evodia odorata* Léveillé enumerates only Cavalerie's no. 2978 and under *Z. odoratum* he enumerates only Cavalerie's no. 1771, though he cites *E. odorata* as synonym. Cavalerie no. 1771 bears inflorescences with flower-buds like Handel-Mazzetti's specimen with which it agrees perfectly; Steward, Chiao and Cheo, no. 708, has ripe fruits.

Orixa japonica Thunberg, Fl. Jap. 61 (1784).—Rehder & Wilson in Sargent, Pl. Wilson. II. 135 (1914).

Sabia Feddei Léveillé in Fedde, Rep. Spec. Nov. IX. 456 (1911); Fl. Kouy-Tchéou, 379 (1915).—**Synon. nov.**

Sabia Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. IX. 456 (1911); Fl. Kouy-Tchéou, 378 (1915).—**Synon. nov.**

Glochidion Vanioti Léveillé in Fl. Kouy-Tchéou, 164 (1914).—**Synon. nov.**

CHINA. Kweichou: Pin-fa, *J. Cavalerie*, no. 22bis, April 4, 1902 "2-3m.; bonne odeur; fleurs vertes" (holotype of *Sabia Feddei;* merotype in A. A.); Pin-fa, *J. Cavalerie*, no. 23bis, April 4, 1902 "petal. 5, vert-jaunes" (holotype of *S. Cavaleriei*, photo. in A. A.); Pin-fa, bois de La-tong, *J. Cavalerie*, no. 575, Oct. 1, 1902 "arbuste" (holotype of *Glochidion Vanioti*, photo. in A. A.).

The specimen of *Sabia Feddei* bears staminate and that of *S. Cavaleriei* pistillate flowers, that of *Glochidion Vanioti* is in fruit.

Boenninghausenia albiflora (Hook.) Reichenbach apud Heynhold, Nomencl. Bot. Hort. I. 126 (1840).[1]—Léveillé, Fl. Kouy-Tchéou, 374 (1915).

Bodiniera thalictrifolia Léveillé & Vaniot in Bull. Acad. Intern. Géog. Bot. XI. 48 (1902).

CHINA. Kweichou: mont de Kao-po (Tsin-gay), haies, herbages de la haute montagne, *J. Laborde* in herb. Bodinier, no. 2702, Nov. 8, 1899 "petales d'un blanc pur" (syntype of *Bodiniera thalictrifolia*, photo. in A. A.); environs de Hoang-ko-chou, haies, buissons, *J. Seguin* in herb. Bodinier, no. 2499, Aug. 1898 "fleurs blanches" (syntype of *Bodiniera thalictrifolia*, photo. in A. A.).

Bodiniera thalictrifolia had been already identified with *Boenninghausenia albiflora* as shown by a note on the sheet of Seguin's specimen. If var. *longipes* Franchet, Pl. Delavay. 123 (1889) is to be considered a distinct variety, the specimens cited above should be referred to that variety, but the difference in the length of the stipe of the carpels seems to be too slight and too gradual to maintain var. *longipes* as distinct. In the typical form the stipe is about 3-4 mm. long and the carpels reach to about the middle of the petals, while in var. *longipes* the stipe is about 5-8 mm. and the carpels are nearer the apex of the petals. The typical form occurs in India and Japan, while the var. *longipes* seems to be the prevailing form in China, except in the southwest where the following variety occurs.

Boenninghausenia albiflora var. α **brevipes** Franchet, Pl. Delavay. 123 (1889).

[1]The binomial is usually credited to Reichenbach, Consp. Reg. Veg. 197 (1828), but at the place Reichenbach published neither a description of the genus nor did he transfer the specific epithet of *Ruta albiflora* Hook. which is cited as a synonym. The first generic description was published in 1836 by Meisner, Pl. Vasc. Gen. 60, and the authority for the generic name therefore should be "Reichenbach apud Meisner."

Boenninghausenia sessilicarpa Léveillé in Fedde, Rep. Spec. Nov. XII. 282 (1913).

Boenninghausenia brevipes (Franch.) Léveillé, Cat. Pl. Yun-Nan, 249 (1917).

CHINA. Yunnan: pâturages des mont. derrière Tong-tchouan, alt. 2550-2700 m., *E. E. Maire,* July 1912 "plante vivace, sous-ligneuse, en touffes dressées" (syntype of *B. sessilicarpa;* photo. in A. A.); pied des mont. vallées de Tong-tchouan, alt. 2500 m., *E. E. Maire,* Aug. 1912 "Ancolie sous-ligneuse, rameuse, petites fleurs blanches" (syntype of *B. sessilicarpa;* photo. in A. A.)

This variety is well distinguished from the type by the nearly sessile carpels, but as there are no concomitant characters except that the leaves are rarely thrice pinnate and the leaflets are smaller, it can hardly be considered specifically distinct. It seems, however, geographically well separated; ten specimens from Yunnan and one from southwestern Szechuan close to the Yunnan border have subsessile carpels except one which like the specimens from all other parts of China has long-stipitate carpels.

Toddalia asiatica (L.) Lamarck, Tab. Encycl. Méth. II. 116 (1793).—Rehder & Wilson in Sargent, Pl. Wilson. II. 137 (1914).

Aralia Labordei Léveillé in Bull. Acad. Géog. Bot. XXIV. 144 (1914); Fl. Kouy-Tchéou, 34 (1914).—**Synon. nov.**

CHINA. Kweichou: environs de Tsin-gay, montagne boisée escarpée près de la ville, *J. Laborde,* Nov. 6, 1898 "arbuste" (holotype of *Aralia Labordei;* merotype in A. A.).

Glycosmis Esquirolii (Lévl.) Tanaka in Bull. Soc. Bot. France, LXXV. 709 (1928); in Tanaka Citrus Exp. Sta. I. no. 2, p. 40 (1932).

Clausena Esquirolii Léveillé in Fedde, Rep. Spec. Nov. IX. 324 (1911); Fl. Kouy-Tchéou, 374 (1915).

CHINA. Kweichou: without locality, *J. Esquirol,* no. 744 (holotype of *Clausena Esquirolii,* photo. in A. A.).

Clausena Dunniana Léveillé in Fedde, Rep. Spec. Nov. XI. 67 (1912).

Clausena Willdenowii Léveillé, Fl. Kouy-Tchéou, 375 (1915); non Wight & Arnott.

CHINA. Kweichou: Pin-fa, rochers, *J. Cavalerie,* no. 1072, June 18, 1903 "petit arbrisseau, fleurs blanches" (holotype; photo. in A. A.).

Léveillé reduced this species in his Flore de Kouy-Tchéou to *C. Willdenowii,* but T. Tanaka in revising Léveillé's specimens maintained the name *C. Dunniana.* Also the determinations by Evans of Handel-Mazzetti's no. 10386 from Kweichou as *C. Dunniana* was confirmed by

T. Tanaka according to his note on the specimen in the Arnold Arboretum herbarium.

Citrus ichangensis Swingle in Jour. Agric. Research. I. 1, fig. 1-7, t. 1 (1913); in Sargent, Pl. Wilson. II. 144 (1914).

? Citrus Cavaleriei Léveillé apud Cavalerie in Bull. Géog. Bot. XXI. 211, 236 (1911), nomen.—Koidzumi, Fl. Symb. Or.-As. 55 (1930).

CHINA. K w e i c h o u : (no specimen in herb. Léveillé).

There are no specimens of *Citrus Cavaleriei* in the Léveillé herbarium and the name was published without description by Cavalerie who states that it is a spiny Orange growing wild at an altitude of 1700 m. near Ma-jo and Kai-tchéou in the province of Kweichou. In the same article Cavalerie publishes a few other manuscript names of *Citrus* given by Léveillé. Léveillé states on p. 236 that W. T. Swingle is inclined to refer *C. Cavaleriei* tentatively to *C. hystrix* DC. Koidzumi, however, identifies it (l. c.) with *C. ichangensis* and makes the latter name a synonym of the former, in spite of the fact that *C. Cavaleriei* is a nomen nudum.

SIMAROUBACEAE

Ailanthus Esquirolii Léveillé, Fl. Kouy-Tchéou, 404 (Sept. 1915), nomen; in Monde Pl. sér. 2, XVII. 23 (Nov. 1915).

CHINA. K w e i c h o u : without locality, *J. Cavalerie*, no. 773 (not now in herb. Léveillé).

Léveillé remarks in Monde des plantes (l. c.) that unfortunately the specimen which had been put aside for a future diagnosis has been mislaid and that only a short diagnosis could be given which runs as follows: "differt ab *A. glutinosa* [*sic*] foliis majoribus conspicue dentatis et floribus coloratis." The plant may not be an Ailanthus at all.

MELIACEAE

Chickrassia tabularis A. Jussieu in Mém. Mus. Paris, XIX. 251 (1830).

Dysoxylon Esquirolii Léveillé in Cat. Pl. Yun-Nan, 176 (1916).—**Synon. nov.**

CHINA. K w e i c h o u : Ycoca-may, *J. Esquirol*, no. 858 (? 898) June 1906 "grand arbre, fleurs jaunes" (holotype of *Dysoxylon Esquirolii;* merotype in A. A.).

The specimen represents a form with the leaves soft-pubescent beneath and sparingly pubescent above; also the rhachis of the leaf and the inflorescence is finely pubescent.

Cipadessa baccifera Miq. var. **sinensis** Rehder & Wilson in Sargent, Pl. Wilson. II. 159 (1914).

Rhus Blinii Léveillé, Fl. Kouy-Tchéou, 411 (1915).—**Synon. nov.**

CHINA. Kweichou: sud de Tin-fan, *J. Cavalerie,* no. 1911, Nov. 1904 (holotype of *Rhus Blinii;* photo. in A. A.).

MALPIGHIACEAE

Aspidopterys Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. IX. 458 (1911); Fl. Kouy-Tchéou, 271 (1914) in part.—Hutchinson in Kew Bull. Misc. Inform. 1917, p. 97.—Niedenzu in Engler, Pflanzenr. IV.-141 (Heft 91) p. 32 (Malpigh.) (1928).

Aspidopterys Dunniana Léveillé in Fedde, Rep. Spec. Nov. XI. 65 (1912); Fl. Kouy-Tchéou, 271 (1914).—Niedenzu, l. c. (1928).

CHINA. Kweichou: Lo-fou, *J. Cavalerie,* no. 2993, April 1908 (holotype of *A. Cavaleriei* and syntype of *A. Dunniana;* photo. in A. A.); Lo-fou, *J. Cavalerie,* no. 3477, March 1909 "fleurs blanches" (syntype of *A. Dunniana;* photo. and fragments in A. A.).

Aspidopterys Dunniana was referred as a synonym to *A. Cavaleriei* by Hutchinson who drew attention to the fact that Léveillé cites Cavalerie no. 2993 under both species, under *A. Dunniana* together with Cavalerie, no. 3477. In his Flore de Kouy-Tchéou, however, Léveillé omits Cavalerie's no. 2993 under *A. Cavaleriei* and cites instead Cavalerie, no. 1882, and Esquirol, no. 712, which do not belong to *Aspidopterys* at all, but represent Combretaceae.

Aspidopterys Esquirolii Léveillé in Fedde, Rep. Spec. Nov. XI. 65 (1912); Fl. Kouy-Tchéou, 271 (1914).—Hutchinson in Kew Bull. Misc. Inform. 1917, p. 100.—Niedenzu in Engler, Pflanzenr. IV.-141 (Heft 91) p. 21 (Malpigh.) (1928).

Cavalierella cordata Léveillé, Fl. Kouy-Tchéou, 61 (1914).—**Synon. nov.**

CHINA. Kweichou: without locality, *J. Esquirol,* no. 593, Aug. 1905 "arbrisseau, fleur jaune" (ex Léveillé et Hutchinson; syntype); Houa-kiang, *J. Cavalerie,* no. 2032, June 6, 1904 (syntype; photo. in A. A.); Gan-chouen, *J. Cavalerie,* no. 3961, May 1912 (holotype of *Cavalierella cordata;* photo. and merotype in A. A.).

This species is according to Hutchinson easily distinguished from the other species by the externally densely hairy sepals.

Esquirol's no. 593 I have not seen, but it is cited by Hutchinson and apparently does not differ from Cavalerie's 3961.

Cavalierella cordata Lévl. one of the two species on which Léveillé based the new genus *Cavalierella* placed into the Caprifoliaceae is undoubtedly an *Aspidopterys* and seems referable to *A. Esquirolii* except that the leaves are subcordate to cordate at the base and generally broader and larger than in Cavalerie's no. 3961; the specimen is in fruit and the fruit is suborbicular, about 3 cm. broad and slightly longer,

cristate between the wings which are furnished with setose hairs except near the margin. The other species of *Cavalierella, C. Dunniana,* belongs to *Dipelta.*

EUPHORBIACEAE

Andrachne Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XII. 187 (1913); Fl. Kouy-Tchéou, 158 (1914).

Andrachne hypoglauca Léveillé, l. c. (1913); l. c. (1914).

China. Kweichou: montagnes de Lou-tsong-koan, rocailles, talus pierreux, *E. Bodinier,* no. 1662, July 12, 1897 "petit arbuste" (holotype of *A. Bodinieri,* merotype in A. A.); bord de la plaine de Tou-chan, *J. Cavalerie,* July 16, 1897 (holotype of *A. hypoglauca;* photo. in A. A.).

This species seems nearest to *A. chinensis* Bge., but differs in its narrower, oblong to lanceolate glabrous leaves, cuneate at the base, with revolute margin and of chartaceous texture with the midrib and lateral veins impressed above and prominent beneath. In the type of *A. hypoglauca* the leaves are narrower and more glaucous beneath than in *A. Bodinieri.* In the shape of the leaves it resembles *A. lolonum* Hand.-Mazz., but this has the leaves densely pubescent beneath.

This and the other species of *Andrachne* described by Léveillé are only mentioned by name as species dubiae by Pax and Hoffmann, in Engler, Pflanzenr. IV.-147, XXV. p. 178 (1922).

Andrachne Esquirolii Léveillé in Fedde, Rep. Spec. Nov. IX. 327 (1911); Fl. Kouy-Tchéou, 158 (1914).

Andrachne persicariifolia Léveillé in Fedde, Rep. Spec. Nov. XI. 187 (1913).

Andrachne attenuata Handel-Mazzetti in Akad. Anz. Wiss. Wien, 1921, p. 178 (Pl. Nov. Sin. Forts. 13, p. 2) (1921). **Synon. nov.**

China. Kweichou: without locality, *J. Esquirol,* no. 110 (holotype of *A. Esquirolii;* photo. in A. A.); environs de Kouy-yang; bois de la pagoda de Kien-lin-chan, dans les rocailles, "item au Ke-ma-tong," *E. Bodinier,* no. 1695, July 20, 1897 (holotype of *A. persicariifolia,* photo. in A. A.); Djitschangping prope oppidum Muyu ad austr.-occ. oppidi Dschenning, *Handel-Mazzetti,* no. 10402, June 22, 1917 (holotype of *A. attenuata;* isotype in A. A.).

In the shape of its leaves and other characters *A. Esquirolii* resembles closely the type of *A. attenuata,* while *A. persicariifolia* has much narrower leaves varying from oblong-lanceolate to narrow-lanceolate, but in the acute sepals and the strigose-setose ovary it agrees with *A. attenuata.*

Securinega suffruticosa (Pall.) Rehder in Jour. Arnold Arb. XIII. 338 (1932).

Securinega ramiflora (Ait.) Mueller Arg. in De Candolle, Prodr. XV. pt. I. 449 (1866).

Securinega fluggeoides Mueller Arg., l. c. 550 (1866).

Phyllanthus Argyi Léveillé in Mem. Acad. Ci. Barcelona, XII. 550 (Cat. Pl. Kiang-Sou, 10) (1916).—**Synon. nov.**

CHINA. Kiangsu: without locality, *d'Argy*, no. 78, (1846-66) "arbrisseau" (holotype of *Phyllanthus Argyi;* merotype in A. A.)

Phyllanthus emblica Linnaeus, Spec. Pl. 982 (1753).

Phyllanthus Mairei Léveillé in Bull. Géog. Bot. XXV. 23 (1915); Cat. Pl. Yun-Nan, 97 (1916).—**Synon. nov.**

CHINA. Yunnan: rives du fleuve Bleu, à Siao-ho, alt. 400 M., *E. E. Maire*, May 1912 "arbrisseau toujours vert; fleurs jaunes; fruits verts, acides, en forme de cerise" (holotype of *P. Mairei*, merotype in A. A.).

Phyllanthus Mairei has been already identified by W. W. Smith with *P. emblica* according to a note on the sheet.

Phyllanthus Franchetiana Léveillé in Bull. Géog. Bot. XXV. 23 (1915); Cat. Pl. Yun-Nan, 97 (1916).

CHINA. Yunnan: river du fleuve Bleu, à Siao-ho, alt. 400 m., *E. E. Maire*, May 1912 "fleurs rougeâtres, drupes vertes, acidulées, en forme de cerise" (holotype; photo. and fragments in A. A.).

This species resembles *P. pulcher* Wall., but the leaves which are 6-8 mm. long are not mucronate and the sepals are crenate-dentate rather than laciniate. The fruits which are not present on the specimen are cherry-like according to the collector's note.

Phyllanthus Dunnianus (Lévl.) Handel-Mazzetti in herb.

Phyllanthodendron Dunnianum Léveillé in Fedde, Rep. Spec. Nov. IX. 324 (1911); Fl. Kouy-Tchéou, 166 (1914).—Handel-Mazzetti, Symb. Sin. VII. 224 (1931).

Phyllanthodendron Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. IX. 454 (1911); Fl. Kouy-Tchéou, 116 (1914).—**Synon. nov.**

Phyllanthodendron Dunnianum Lévl. var. *hypoglaucum* Léveillé, Fl. Kouy-Tchéou, 166 (1914).

CHINA. Kweichou: Lo-fou, *J. Cavalerie*, no. 2659, Nov. 1905 "fl. vertes" (holotype of *Phyllanthodendron Dunnianum;* merotype in A. A.); O-to près Lo-fou, *J. Cavalerie*, no. 3284, April 1907 "arbrisseau" (holotype of *Ph. Cavaleriei;* merotype in A. A.); Lo-fou, *J. Cavalerie*, no. 3500, April, May 1909 (holotype of *Ph. Dunnianum* var. *hypoglaucum;* merotype in A. A.).

The three specimens cited above agree in the distinctly winged branchlets and generally in the shape of the leaves, but differ in several other characters. In *Phyllanthodendron Dunnianum* var. *hypoglaucum* (Cavalerie, no. 3500) which is in fruit the leaves are glaucous beneath and the branchlets are pilose, while in the other two specimens they are

perfectly glabrous. *Phyllanthodendron Dunnianum* (Cavalerie, no. 2659) has thin membraneous leaves, while *Ph. Cavaleriei* (Cavalerie, no. 3284) has subcoriaceous leaves with prominent veins on both sides; both specimens are in flower. Cavalerie's nos. 3284 and 3500 have dimorphic leaves, while no. 2659 which consists only of two small sterile branchlets (with a detached flower in a pocket) has only one kind of leaves. The specimen of no. 2659 bears in Handel-Mazzetti's handwriting the new combination "*Phyllanthus Dunnianus* (Lévl.)" but in his remarks on *Phyllanthodendron Dunnianum* under his *Phyllanthus anthopotamicus* (in Symb. Sin. VII. 224. 1931) he does not cite this combination; he only states that the two species are closely related. *Phyllanthus Dunnianus*, however, differs markedly from *P. anthopotamicus* in the strongly angular, slightly winged glabrous or only slightly pilose branchlets, the quite glabrous, more acuminate leaves dark green above and generally larger, and in the larger, longer-stalked glabrous flowers.

Phyllanthus spec.

Sterculia Bodinieri Léveillé, Fl. Kouy-Tchéou, 406 (1915).—**Synon. nov.**

CHINA. K w e i c h o u : environs de Hoang-ko-chou, grande cascade, au bord de l'eau, *J. Seguin* in herb. Bodinier, no. 2194, April 1898 "arbuste, fleurs rougeâtres" (holotype of *Sterculia Bodinieri;* merotype in A. A.).

This plant is undoubtedly a Euphorbiacea and seems referable to the sect. Eriococcus of *Phyllanthus* except that it has 4 anthers.

Glochidion puberum (L.) Hutchinson in Sargent, Pl. Wilson. II. 518 (1916).

Glochidion Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XII. 183 (1913); Fl. Kouy-Tchéou, 163 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : Kouy-yang, mont du Collège, *E. Bodinier*, no. 2307, July 21, 1897 (fruit), June 9, 1898 "arbuste de 1 m., très branchu" (holotype of *G. Bodinieri;* merotype in A. A.).

Glochidion villicaule Hooker f., Fl. Brit. Ind. v. 326 (1887).

Glochidion Esquirolii Léveillé in Fedde, Rep. Spec. Nov. XII. 186 (1913); Fl. Kouy-Tchéou, 163 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : Ouang-mou, *J. Esquirol,* no. 714, June 1904 "fleurs jaunes" (holotype of *G. Esquirolii;* photo. in A. A.).

Baccaurea Cavaleriei Léveillé, Fl. Kouy-Tchéou, 159 (1914).

CHINA. K w e i c h o u : Lo-fou, *J. Cavalerie,* no. 3299, April 1907 "petit arbre" (holotype, photo. in A. A.).

In the general habit and in the leaf this species has some resemblance to *B. sapida* Muell. Arg., but the material is insufficient for exact

determination; it consists of a leafy branch and broken fragments of immature inflorescences.

Antidesma microphyllum Hemsley in Jour. Linn. Soc. Bot. xxvi. 432 (1894).—Handel-Mazzetti, Symb. Sin. vii. 218 (1931).

Antidesma Seguini Léveillé in Fedde, Rep. Spec. Nov. ix. 460 (1911); Fl. Kouy-Tchéou, 158 (1914).—Pax & Hoffmann in Engler, Pflanzenr. iv.-147, xv. 166 (1922).

Myrica Darrisii Léveillé in Fedde, Rep. Spec. Nov. xii. 537 (1913); Fl. Kouy-Tchéou, 281 (1914).—**Synon. nov.**

China. Kweichou: district de Tchen-lin, bord du fleuve à la cascade de Hoang-ko-chan, *J. Seguin,* June 10, 1898 "fleur blanche" (ex Léveillé; syntype of *A. Seguini*); fleuve Hoa-kiang, *J. Esquirol,* no. 505, June 1905; (ex Léveillé; syntype of *A. Seguini*); Tchai-chouï-ho, *J. Esquirol,* no. 1586, July 1909 (syntype of *A. Seguini;* merotype in A. A.); route de Mou-you-se à Houang-ko-chou, *J. Cavalerie,* no. 2058, June 10, 1904 (holotype of *Myrica Darrisii;* photo. and fragment in A. A.).

Of the three syntypes of *A. Seguini* I have before me only Esquirol, no. 1568, which agrees exactly with Henry's 9530a of *A. microphyllum* Hemsl., while Cavalerie, no. 2058, the holotype of *Myrica Darrisii,* differs slightly in, at least partly, somewhat broader leaves. *Antidesma Seguini* had been identified with *A. microphyllum* by Dr. Stapf according to a note in a letter from Dr. Handel-Mazzetti of Dec. 25, 1922.

Croton Tiglium Linnaeus, Spec. Pl. 1004 (1753).—Handel-Mazzetti, Symb. Sin. vii. 218 (1931).

Alchornea Vanioti Léveillé, Cat. Pl. Yun-Nan, 95 (1916).

China. Yunnan: Tong-tchouan, *E. E. Maire,* 1911 (holotype of *Alchornea Vanioti;* merotype in A. A.).

Alchornea Vanioti was identified with *Croton Tiglium* by Handel-Mazzetti (l. c.).

Speranskia cantonensis (Hance) Pax & Hoffmann in Engler, Pflanzenr. iv.-147, vi. 15 (Euphorb.) (1912).

Mercurialis acanthocarpa Léveillé in Fedde, Rep. Spec. Nov. iii. 21 (1906).

Speranskia tonkinensis Léveillé, Fl. Kouy-Tchéou 167 (1914).

China. Kweichou: Pin-fa, près d'une rivière, très rare, *J. Cavalerie,* no. 1585, Oct. 19, 1903 (holotype of *Mercurialis acanthocarpa;* photo. in A. A.).

Mercurialis acanthocarpa was referred by Léveillé together with *Speranskia Henryi* Oliv., to *Speranskia tonkinensis,* a name cited without author and probably a mistake for *S. cantonensis.*

Mallotus Leveillanus Fedde, Rep. Spec. Nov. x. 144 (1912).—Pax & Hoffmann in Engler, Pflanzenr. iv.-147, vii. p. 165 (1914).

Mallotus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. IX. 461 (1911), non Léveillé, l. c. 327.

Mallotus Leveillei Fedde apud Léveillé, Fl. Kouy-Tchéou, 165 (1914).

China. Kweichou: Ouang-mou, *J. Esquirol*, no. 120, June 1904 (holotype of *M. Esquirolii;* photo. in A. A.); Lo-fou, *J. Cavalerie*, no. 3666, Aug. 1909 (duplicate in A. A.).

Cavalerie's no. 3666 is cited together with Esquirol's no. 120 by Léveillé under *M. Leveillei* (l. c.) and by Pax & Hoffmann under *M. Leveillanus* (l. c.).

Mallotus philippinensis (Lam.) Mueller Arg. in Linnaea, XXXIV. 196 (1865).—Pax & Hoffmann in Engler, Pflanzenr. IV.-147, VII. p. 184 (1914).

Evonymus hypoleucus Léveillé in Fedde, Rep. Spec. Nov. XIII. 260 (1914).—**Synon. nov.**

Phyllanthodendron sp. Léveillé, Fl. Kouy-Tchéou, 166 (1914).

China. Kweichou: Lo-fou, *J. Cavalerie*, no. 2733, April 1906 (holotype of *Evonymus hypoleucus* and *Phyllanthodendron* sp.; photo. in A. A.).

Evonymus hypoleucus is cited by Léveillé in his Flore de Kouy-tchéou as a synonym of *Phyllanthodendron* sp.

Mallotus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. IX. 327 (1911); Fl. Kouy-Tchéou, 165 (1914).—Pax & Hoffmann in Engler Pflanzenr. IV.-147, VII. 196 (1914).

China. Kweichou: without precise locality, *J. Esquirol*, no. 898 (holotype; photo. and fragments in A. A.); Lo-fou, *J. Cavalerie*, no. 3114, March 1909 (cited in Fl. Kouy-Tchéou; duplicate in A. A.).

Mallotus Milliettii Léveillé, Fl. Kouy-Tchéou, 165 (1914).

China. Kweichou: route de Gan-chouen à Hin-y-fou, *Cavalerie*, no. 3967, July 1912 (holotype; photo. and fragments in A. A.).

This species seems nearest to *M. contubernalis* Hance, but differs chiefly in the leaves being sparingly stellate-pubescent above and rather densely and softly so below, and in the subsessile larger capsules about 1.4 cm. in diam. with a heavier indumentum. In the pubescence of the leaves it seems to approach var. *chrysocarpus* (Pamp.) Hand.-Mazz. which I have not seen, the type of it being missing from the Herb. Biondi when I was in Florence in 1932.

Léveillé cites Cavalerie, no. 3697, as the type of his species, but the specimen is numbered 3967 and bears in Léveillé's handwriting the name *Mallotus Cavaleriei* in ink and below in pencil *M. Milliettii*. Léveillé apparently changed the name *M. Cavaleriei* which was never published for this plant, to *M. Milliettii*, because he had already given

the former name to a species which was later referred to *Discocleidion rufescens* (Fr.) Pax & Hoffm.

Discocleidion rufescens (Franch.) Pax & Hoffmann in Engler, Pflanzenr. IV.-147, VII. p. 45, fig. 6 (1914).—Léveillé, Fl. Kouy-Tchéou, 161 (1914).

Mallotus Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. XI. 296 (1912).

CHINA. Kweichou: Gan-chouen, *J. Cavalerie,* no. 3825, June 1910 (holotype of *Mallotus Cavaleriei;* photo. in A. A.).

Acalypha Mairei (Lévl.) Schneider in Sargent, Pl. Wilson. III. 301 (1916).—Léveillé, Cat. Pl. Yun-Nan, 94 (1916).—Pax & Hoffmann in Engler, Pflanzenr. IV.-147, XVI. 137 (1924).

Morus Mairei Léveillé in Fedde, Rep. Spec. Nov. XIII. 265 (1914).

CHINA. Yun-nan: brousse derrière Mo-tsou fleuve Bleu, alt. 800 m., *E. E. Maire,* May (ex Léveillé; syntype of *Morus Mairei*); rochers de Ma-hong, alt. 3000 m., *E. E. Maire,* June 1912 (syntype of *Morus Mairei,* merotype in A. A.).

Tragia involucrata Linnaeus, Spec. Pl. 980 (1753).—Pax & Hoffmann in Engler, Pflanzenr. IV.-147, IX-XI. 81 (1919).—Handel-Mazzetti, Symb. Sin. VII. 218 (1931).

Alchornea Mairei Léveillé, Cat. Pl. Yun-Nan, 94 (1916).

CHINA. Yunnan: vallon de Yon-fong-keou, alt. 800 m., *E. E. Maire,* July 1912 (holotype of *Alchornea Mairei;* photo. in A. A.).

Alchornea Mairei was first identified with *Tragia involucrata* by Handel-Mazzetti who states that it approaches var. *intermedia* Muell. Arg.

Sapium rotundifolium Hemsley in Jour. Linn. Soc. Bot. XXVI. 445 (1894).—Handel-Mazzetti, Symb. Sin. VII. 212 (1931).

Baccaurea Esquirolii Léveillé, Fl. Kouy-Tchéou 159 (1914).—**Synon. nov.**

CHINA. Kweichou: Mou-you-se, *J. Cavalerie,* no. 2137 (no. 13), June 1904 "arbre, fl. jaunes" (syntype of *Baccaurea Esquirolii;* photo. in A. A.); Lo-fou, *J. Cavalerie,* no. 3458, Oct. 1908 (syntype of *B. Esquirolii;* photo. in A. A.); without precise locality, *J. Esquirol,* no. 517, June 1905 (syntype of *B. Esquirolii*; photo. in A. A.).

This species was collected in Kweichou also by Handel-Mazzetti (nos. 10279 and 10371).

DAPHNIPHYLLACEAE

Daphniphyllum macropodum Miquel in Mus. Bot. Lugd.-Bat. III. 129 (1867).—Rosenthal in Engler, Pflanzenr. IV.-147a, p. 9 (1919).

Webera Marchandii Léveillé in Fedde, Rep. Spec. Nov. XIII. 178 (1911); Fl. Kouy-Tchéou, 372 (1915).—**Synon. nov.**

China. Kweichou: moulins de Tong-tchéou, *J. Esquirol* & *R. Marchand,* no. 3252, June 22, 1912 (holotype of *Webera Marchandii;* photo. in A. A.).

BUXACEAE

Sarcococca Hookeriana Baill. var. **humilis** Rehder & Wilson in Sargent, Pl. Wilson. II. 164 (1914).

Maesa spec. Léveillé, Fl. Kouy-Tchéou, 287 (1914).

Myrsine Chevalieri Léveillé, Fl. Kouy-Tchéou, 287 (1914).— **Synon. nov.**

Pachysandra Mairei Léveillé, Cat. Pl. Yun-Nan, 97, fig. 23 (1916).

China. Kweichou: enfoncement de Ouan-ly près Thou-ly, *J. Esquirol,* no. 2593, Feb. 1911, "fleurs jaunâtres, fruits rouges" (holotype of *Myrsine Chevalieri;* merotype in A. A.). Yunnan: au pied de rochers, collines arides à l'est de Tong-tchouan, alt. 2600 m., *E. E. Maire,* March 1912 "arbrisseau toujours vert en touffes; fleurs blanches; fruits noirs" (holotype of *Pachysandra Mairei;* merotype in A. A.).

Esquirol's no. 2593, *Myrsine Chevalieri,* agrees exactly with specimens of *Sarcococca Hookeriana* var. *humilis* and I have no doubt that it belongs here, though Esquirol states that it has red fruits; the specimen before me bears only immature inflorescences. Under *Myrsine Chevalieri* Léveillé cites (l. c. p. 288) *Maesa* spec., Esquirol 2593, appearing on the preceding page as being the same. *Pachysandra Mairei* was identified by Handel-Mazzetti (Symb. Sin. VII. 235) with *S. Hookeriana* var. *digyna,* but I think it belongs with var. *humilis* which, however, may be only a dwarf and smaller form of var. *digyna.* A topotype or perhaps an isotype of *P. Mairei* was distributed as *Sarcococca* spec. E. E. Maire, no. 355, by the Arnold Arboretum.

Pachysandra stylosa Dunn in Jour. Bot. XLVI. 326 (1908).— Handel-Mazzetti, Symb. Sin. 236 (1931).

Pachysandra Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XII. 187 (1913).

Pachysandra axillaris Franch. var. *Kouytchensis* Léveillé, Fl. Kouy-Tchéou, 166 (1914).

China. Kweichou: monts entre Ma-kay et Se-tchong-hien à Tien-sen-kiao, rochers à l'entrée du Tien-sen-kiao, *E. Bodinier,* no. 1525, Aug. 5, 1897 (holotype of *P. Bodinieri;* photo. in A. A.); Tsingay, *E. Bodinier* (holotype of *P. axillaris* var. *Kouytchensis;* photo. in A. A.).

Pachysandra Bodinieri is not enumerated in the Flore de Kouy-Tchéou, nor is Bodinier's no. 1525 cited under *Pachysandra.* According to Handel-Mazzetti both belong to *P. stylosa.*

Buxus microphylla S. & Z. var. **aemulans** Rehder & Wilson in Sargent, Pl. Wilson. II. 169 (1914).

Buxus Bodinieri in Fedde, Rep. Spec. Nov. XI. 549 (1913); Fl. Kouy-Tchéou, 160 (1914).—**Synon. nov.**

China. K w e i c h o u : environs de Kouy-yang, mont du Collège, *E. Bodinier,* no. 2079, Feb. 25, 1898 "arbuste plus ou moins grand" (holotype of *B. Bodinieri;* merotype in A. A.).

The filaments in Bodinier's plant are longer than in the type of var. *aemulans.*

Buxus megistophylla Léveillé, Fl. Kouy-Tchéou, 160 (1914); Cat. Ill. Pl. Seu-Tchouen, pl. 26 (1918).

China. K w e i c h o u : environs de Hoang-ko-chou, rocailles de la cascade, *J. Seguin* in herb. Bodinier, no. 2607, March 15, 1899 "arbuste de 0.60 cm., fleurs blanches" (syntype; merotype in A. A.); Kiao-men, près Lo-fou, *J. Esquirol,* no. 2560, Dec. 1910 (syntype; photo. in A. A.).

In its large leaves and subterete branchlets *B. megistophylla* resembles *B. Henryi* Mayr, but differs in the glabrous inflorescence, subsessile staminate flowers (in *B. Henryi* on pedicels 2-3 mm. long) and short stigmas; from *B. microphylla* S. & Z. it differs besides in the large leaves, chiefly in the short rudimentary ovary and the long filaments of the staminate flower.

Buxus Myrica Léveillé in Fedde, Rep. Spec. Nov. XI. 549 (1913); Fl. Kouy-Tchéou, 160 (1914).

Frutex ramulis tetragonis breviter pilosis gracilibus. Folia brevissime petiolata petiolis pilosulis, oblongo-lanceolata vel anguste lanceolata, 2-5 cm. longa et 0.5-1.4 cm. lata, acuta, mucronulata, basi cuneata, glabra, tenuiter coriacea et in sicco utrinque distincte reticulata, costa media utrinque elevata. Racemi axillares numerosi, rhachi dense pilosula elongata 5-7 mm. longa; bractearum paria 6-8, bracteae ovatae, acutae, 2 mm. longae, dorso dense pilosulae et intus ad marginem villosulis; flores ♂ breviter pedicellati pedicello 1-1.5 mm. longo dense pilosulo, sepalis ovalibus, 2 exterioribus carinatis carina ciliata, 2 interioribus longioribus glabris circ. 3 mm. longis, staminibus sepala superantibus 4 mm. longis, rudimento ovarii sepalis triplo breviore; flos ♀ terminalis, sepalis oblongo-ovatis 3-4 mm. longis, 3 exterioribus dorso dense 3 interioribus sparse pilosulis, ovario stylis complanatis apice recurvis multo breviore (ovarium 1.5 mm. longum, styli 3.5 cm. longi), stigmatibus ad medium stylum decurrentibus. Fructus non visus.

China. K w e i c h o u : Pin-fa, *J. Cavalerie,* no. 3198, April 8, 1907 (syntype; merotype in A. A.); Lo-hou, *J. Esquirol,* no. 2566, Dec. 1910 (ex Léveillé; syntype); grande cascade de Hoang-ko-chou, dans

les rochers, *J. Seguin* in herb. Bodinier, no. 2266, April 3, 1898 (syntype; photo. in A. A.).

This species, of which I have given a description above, since that of Léveillé is inadequate, seems most closely related to *B. Henryi* Mayr, but differs in the quadrangular pilose branchlets, narrower and smaller thinly coriaceous and reticulate leaves, smaller and acute densely pilose bracts not scarious on the margin, in the shorter pedicels of the staminate flowers (in *B. Henryi* 2-3 mm. long) shorter filaments and shorter flattened styles. The two specimens which I have seen differ slightly in the size and shape of the leaves; Cavalerie's no. 3198 has the leaves mostly 3-5 cm. long and up to 1.4 cm. broad, while Bodinier's no. 2266 has comparatively narrower leaves 2-5 cm. long (a few even smaller) and 5-10 mm. broad; in the former the staminate flowers have mostly dropped while the latter is in full bloom.

Buxus Harlandi Hance var. **cephalantha** (Lévl. & Vant.), comb. nov.

Buxus cephalantha Léveillé & Vaniot in Fedde, Rep. Spec. Nov. III. 21 (1906).

Buxus sempervirens var. *microphylla* Léveillé, Fl. Kouy-Tchéou, 160 (1914).—Non Siebold & Zuccarini.

China. Kweichou: Pin-fa, rochers dans ou près ruisseaux, *J. Cavalerie,* no. 1797, Aug. 25, 1904 "tout petit buis, 1 pied de h." (holotype of *B. cephalantha;* photo. in A. A.).

This form differs from the type chiefly in its very small size; the plant is about 30 cm. high with short branchlets, the leaves measure 6-11 mm. in length and are mostly slightly emarginate and partly obovate (8:4 mm.), the inflorescences are mostly terminal.

CORIARIACEAE

Coriaria sinica Maximowicz in Mém. Acad. Sci. St. Pétersb. sér. 7, XXIX. no. III. 9, fig. (1881).—Léveillé Cat. Pl. Yun-Nan, 249 (1917).

Morus calva Léveillé in Fedde, Rep. Spec. Nov. XIII. 265 (1914).

China. Yunnan: coteaux arides à La-kou, alt. 2400 m., *E. E. Maire,* March 1912 "grand arbuste a l'écorce fibreuse, fleurs jaunâtres, d'abord rouges" (holotype of *Morus calva;* merotype in A. A.).

Morus calva was first identified with *Coriaria sinica* by C. Schneider (in Sargent, Pl. Wilson. III. 301. 1916).

ANACARDIACEAE

Spondias axillaris Roxburgh, Cat. Hort. Beng 34 (1814), nomen; Fl. Ind. II. 453 (1832).—Rehder & Wilson in Sargent, Pl. Wilson. II. 172 (1914).

Rhus Bodinieri Léveillé in Fedde, Rep. Spec. Nov. x. 437 (1912).—**Synon. nov.**

CHINA. Hongkong: bois de Happy Valley; bord de la routs à Pok-fu-lum, *E. Bodinier*, no. 1103, April 6 & 15, 1895 (holotype of *Rhus Bodinieri;* photo. in A. A.).

Pistacia chinensis Bunge in Mém. Div. Sav. Acad. Sci. St. Pétersb. II. 89 (Enum. Pl. Chin. Bor. 15) (1833).—Rehder & Wilson in Sargent, Pl. Wilson. II. 173 (1914).

Rhus gummifera Léveillé in Fedde, Rep. Spec. Nov. x. 474 (1912); Fl. Kouy-Tchéou, 412 (1915).—**Synon. nov.**

Rhus Argyi Léveillé in Mem. Acad. Ci. Barcelona, ser. 3, XII. 562 (Cat. Pl. Kiang-Sou, 22) (1916).—**Synon. nov.**

CHINA. Kiangsu: without precise locality, *Ch. d'Argy*, (1846-66) (holotype of *Rhus Argyi;* merotype in A. A.). Kweichou: au sud de Pin-fa près ruisseau, sur rochers, *J. Cavalerie*, no. 1078, June 18, 1903 "arbre produisant une espèce de gomme odorante" (holotype of *Rhus gummifera;* photo. and fragments in A. A.).

The specimen from Kiangsu has the rachis and the midrib of the leaflets below densely and finely pubescent while that from Kweichou is nearly glabrous. This in general seems to constitute a difference between the eastern and the western plants of this species; the specimens before me from the eastern provinces are mostly more or less pubescent on the rachis and the midrib of the leaflets, while the western specimens are glabrous or nearly so.

Rhus punjabensis Stewart var. **sinica** (Diels) Rehder & Wilson in Sargent, Pl. Wilson. II. 176 (1914).

? Rhus echinocarpa Léveillé in Fedde, Rep. Spec. Nov. x. 475 (1912); Fl. Kouy-Tchéou, 411 (1915), pro parte, quoad specim. Cavalerie, no. 2003.

Rhus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. XII. 181 (1913); Fl. Kouy-Tchéou, 411 (1915).—**Synon. nov.**

? Rhus Mairei Léveillé, Sert. Yunnan. 2 (1916); Cat. Pl. Yun-Nan, 269 (1917).—**Synon. nov.**

CHINA. Kweichou: Tsin-gai, bois, *J. Cavalerie*, no. 1157, July 13, 1903, "petit arbre, fruit rouge-velour" (holotype of *Rh. Esquirolii;* photo. in A. A.). Yunnan: pied de montagne, derrière Tong-tchouan, alt. 2550 m., *E. E. Maire*, May 1912 "petit arbre cassant, rameaux rares, fleurs blanches" (syntype of *Rh. Mairei;* merotype in A. A.); haies de la plaine à Tong-tchouan, alt. 2500 m., July 1912 "fruit d'un petit arbre cassant, fl. blanches en grappes," *E. E. Maire* (syntype of *Rh. Mairei;* photo. in A. A.); plaine de Tche-hai, haies, alt. 2500 m., *E. E. Maire*, Aug. 1912 (ex Léveillé; syntype of *Rh. Mairei*).

Rhus Mairei I refer here with some doubt; it has partly only 5-7 leaflets and the rachis is not or very slightly winged, but otherwise it

seems to agree with *Rh. punjabensis* var. *sinica.* I also refer here doubtfully Cavalerie's no. 2003 numerated by Léveillé as a syntype of *Rh. echinocarpa.*

Rhus trichocarpa Miquel in Ann. Mus. Bot. Lugd.-Bat. II. 84 (1866); Prol. Fl. Jap. 16 (1866).—Rehder & Wilson in Sargent, Pl. Wilson. II. 180 (1914).

Rhus echinocarpa Léveillé in Fedde, Rep. Spec. Nov. x. 475 (1912); Fl. Kouy-Tchéou, 411 (1915); specim. no. 2003 excluso.—**Synon. nov.**

CHINA. Kweichou: Mou-you-se, *J. Cavalerie,* no. 1016 (flowers), May 28, 1903 (syntype of *Rh. echinocarpa;* photo. and fragments in A. A.); without precise locality, fruit, *J. Cavalerie,* no. 108 (2) [?] (photo. in A. A.).

Under *Rhus echinocarpa* Léveillé cites two specimens, nos. 1016 and 2003, of which only the first belongs here, while the second is identical with *Rhus punjabensis* var. *sinica;* both are flowering specimens. The description of the fruit, from which the name of the species is derived, is apparently based on an unnamed specimen in the Léveillé herbarium marked only "J. Cavalerie, 108 (2)" on a slip and placed in the same cover with the other two specimens. As there is no other fruiting specimen of *Rhus* in the Léveillé herbarium which answers to the description of the very characteristic fruit of this species, this must be the specimen from which the description of the fruit was drawn, though it is not cited.

This record extends the range of *Rh. trichocarpa* west into Kweichou.

AQUIFOLIACEAE

Ilex suaveolens (Lévl.) Loesener in Ber. Deutsch. Bot. Ges. XXXII. 541 (1914).—Léveillé, Fl. Kouy-Tchéou, 201 (1914).

Celastrus suaveolens Léveillé in Fedde, Rep. Spec. Nov. XIII. 263 (1914).

CHINA. Kweichou: environs de Kouy-yang, bois de Kien-lin-chan, *E. Bodinier,* no. 2663 [not 2683], June 19, 1899, "arbre de haute taille" (syntype of *Celastrus suaveolens;* photo. and fragments in A. A.); Pin-fa, *J. Cavalerie,* no. 17bis, June 3, 1902 "arbrisseau 5-6 m., fleurs blanches parfumées" (syntype of *C. suaveolens;* fragments in A. A.).

A full description of the species is given by Loesener (l. c.).

Ilex purpurea Hasskarl, Cat. Pl. Bogor. 230 (1844).—Loesener in Nov. Act. Leop.-Carol. Akad. LXXVIII. 111 (Monog. Aquifol.) (1901).

Callicarpa Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. IX. 455 (1911); Fl. Kouy-Tchéou, 439 (1915).—**Synon. nov.**

Embelia rubro-violacea Léveillé in Fedde, Rep. Spec. Nov. x. 375 (1912); Fl. Kouy-Tchéou, 285 (1914).—**Synon. nov.**

Celastrus Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XIII. 263 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : environs de Tou-chan, *J. Cavalerie*, no. 2624, June 3, 1899 "grand arbuste, fleurs roses" (holotype of *Callicarpa Cavaleriei;* photo. in A. A.); Pin-fa, *J. Cavalerie*, no. 1334, June 23, 1902 (ex Léveillé; syntype of *Embelia rubro-violacea*); forêts, *J. Esquirol*, no. 429, June 1905, "grand arbrisseau" (syntype of *E. rubro-violacea;* merotype in A. A.); environs de Kouy-yang, mont du Collège, *E. Bodinier*, no. 2384, June 10, 1898 "arbuste" (holotype of *Celastrus Bodinieri;* photo. in A. A.).

The specimens cited above belong to var. *Oldhami* (Miq.) Loes. which represents the type of *I. purpurea* Hassk.; all are staminate plants in bloom.

Ilex pedunculosa Miq. var. **continentalis** Loesener in Nov. Act. Leop.-Carol. Akad. LXXVIII. 110 (Monog. Aquifol.) (1901); in Sargent, Pl. Wilson. I. 76 (1911).

Ilex purpurea var. δ *Leveilleana* Loesener in Léveillé, Fl. Kouy-Tchéou, 201 (1914).

CHINA. K w e i c h o u : Pin-fa, bois, *J. Cavalerie*, no. 1066, June 1903 (holotype of *I. purpurea* var. *Leveilleana;* photo. in A. A.).

Cavalerie's no. 1066 was considered by Loesener according to a note by him on the sheet of the type specimen, a possible hybrid between *I. purpurea* Hassk. and *I. pedunculosa* Miq., but the specimen seems to agree well in all respects with *I. pedunculosa* except that the petioles are not as deeply channelled as in *I. pedunculosa* and the margins of the leaves are somewhat more serrulate. In the solitary flowers (or in one case a 3-flowered cyme) and in the texture of the leaves it certainly agrees with *I. pedunculosa* which often has slightly serrulate leaves; the midrib above is short-pilose as it is, though usually to a lesser degree, in *I. pedunculosa;* in *I. purpurea* it is perfectly glabrous.

Ilex metabaptista Loes. var. **myrsinoides** (Lévl.), var. nov.

Maesa myrsinoides Léveillé in Fedde, Rep. Spec. Nov. X. 375 (1912); Fl. Kouy-Tchéou, 286 (1914).

Myrsine Feddei Léveillé in Fedde, Rep. Spec. Nov. X. 376 (1912); Fl. Kouy-Tchéou, 288 (1914).—**Synon. nov.**

Embelia Cavaleriei Léveillé, Fl. Kouy-Tchéou, 284 (1914).—**Synon. nov.**

Ilex Fargesii Franch. var. *Bodinieri,* Loesener apud Léveillé, l. c. 200 (1914).—**Synon. nov.**

Ilex metabaptista Léveillé, l. c. 200 (1914), vix Loesener.

CHINA. K w e i c h o u : Pin-fa, ruisseau de La-tong, *J. Cavalerie*, no. 579, Oct. 1, 1902 (holotype of *Maesa myrsinoides;* merotype in A. A.); Pin-fa, ruisseaux, *J. Cavalerie*, no. 842, May 23, 1902, "fleurs blanches" (holotype of *Myrsine Feddei;* merotype in A. A.); bords des

ruisseaux et torrents, *J. Cavalerie* in hb. Bodinier no. 2635, June 3, 1899, "fleurs blanches parfumées" (holotype of *Embelia Cavaleriei;* merotype in A. A.); environs de Kouy-yang, Mont du Collège, à la Cascade, *J. Chaffanjon,* May 28, 1898, et environs de Tou-chan, *J. Cavalerie,* June 3, 1899, in herb. Bodinier, no. 2310 (2 sheets, one is the holotype of *I. Fargesii* var. *Bodinieri,* the other represents *I. metabaptista* Lévl., vix Loes.; photos. in A. A.).

This variety differs from the type chiefly in the smaller leaves 2.5-4, rarely up to 6.5 cm. long, shorter 2-4 mm. long petioles and glabrous inflorescences. Of the three specimens cited above, Cavalerie, no. 579, is in fruit, while his no. 842 bears pistillate flowers and no. 2635 staminate flowers; the latter differs in the slightly broader elliptic-oblong leaves perfectly glabrous below, while the other two numbers are like the type sparingly short-pilose on the midrib below chiefly toward the base and pubescent on the midrib above. Bodinier's no. 2310 is represented by two sheets, one containing two staminate specimens, and named by Loesener *I. metabaptista,* while the other with one pistillate specimen is named by Loesener *"I. Fargesii* var. vel forma δ *Bodinieri* Loes. forma nova." The labels on each sheet contain the same information and give two localities, though one sheet has only a single specimen. All three are slightly different. The sheet named *I. metabaptista* by Loesener with two staminate specimens has slightly pilose inflorescences and thus approaching the type of the species, but the pubescence is less dense and the leaves are smaller than in the type. The one named *I. Fargesii* f. *Bodinieri* by Loesener is a pistillate specimen with narrow leaves up to 6.5 cm. long and glabrous inflorescences; on account of the latter character I refer it to var. *myricoides.* From *Ilex Fargesii* to which Loesener referred it, it is at once distinguished by the short petioles which are only half as long as the inflorescence, while in *I. Fargesii* the petioles are 1-1.5 cm. long and exceed the inflorescence, also the leaves are larger and broader and distinctly serrulate to below the middle.

The variety has also been collected in Kweichou by Y. Tsiang (no. 8525) near Tin-fan, Binshaw, and by Steward, Chiao and Cheo (no. 950) near Hung-shieh-lang, Kang-kou-hseh; in the latter specimen some of the leaves are up to 6 cm. long. Tsiang, no. 8525, was first identified by E. D. Merrill with *Maesa myrsinoides* Lévl. and distributed as *I. myrsinoides* (Lévl.) Merr.

Ilex corallina Franchet in Bull. Soc. Bot. France, XXXIII. 452 (1886).—Loesener in Nov. Act. Leop.-Carol. Akad. LXXVIII. 327 (Monog. Aquifol.) (1901).

Ilex Dunniana Léveillé in Fedde, Rep. Spec. Nov. IX. 458 (1911); Fl. Kouy-Tchéou, 200 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : Long-ly, *J. Cavalerie*, no. 3000, May, 1908, "petit arbre, fleurs jaunes" (holotype of *I. Dunniana;* merotype in A. A.).

Cavalerie's specimen represents a form with broad leaves rather closely denticulate with upright-spreading not incurved teeth. Léveillé had recognized the close relation of his species to *I. corallina*, but the differences he gives are either trivial or do not hold.

Ilex corallina Franch. var. **Loeseneri** Léveillé, Fl. Kouy-Tchéou, 200, (1914), nomen.

A typo recedit foliis angustioribus spinuloso-dentatis.

CHINA. K w e i c h o u : Kouy-yang, mont du Collège, *J. Chaffanjon* in herb. Bodinier, no. 2242, Apr. 1898 (syntype; photo. in A. A.); Pin-fa, montagnes, *J. Cavalerie*, no. 580," arbrisseau, fl. odor." (syntype; photo. in A. A.).

The two specimens cited above bear Loesener's identification *"Ilex corallina* Franch. forma" on the sheets without any descriptive note, neither does Léveillé give a description. From the type this variety differs chiefly in the spinulose-serrate narrower leaves; in Cavalerie's no. 580 the spinulose mucro of the teeth is about 1 mm. long, but in Chaffonjon's specimens the mucro is much shorter and the leaves measure 1.5-2 cm. in width, while in Cavalerie's specimen they are 8-10 × 2-2.5 cm.

Ilex macrocarpa Oliver in Hooker, Icon. XVIII. t. 1787 (1888).—Loesener in Nov. Act. Leop.-Carol. Akad. LXXVIII. 489 (1901); Ber. Deutsch. Bot. Ges. XXXII. 543 (1914).—Léveillé, Fl. Kouy-Tchéou, 200 (1914).

Celastrus salicifolia Léveillé in Fedde Rep. Spec. Nov. XIII. 263 (1914).

Diospyros Bodinieri Léveillé, Fl. Kouy-Tchéou, 144 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : without precise locality, *J. Esquirol*, May 10, 1906 "arbre" (holotype of *Celastrus salicifolia;* photo. in A. A.); Kouy-yang, mont du Collège, *J. Chaffanjon* in herb. Bodinier, April 1898 "arbre" (holotype of *Diospyros Bodinieri;* merotype in A. A.).

Celastrus salicifolia was first identified with *Ilex macrocarpa* by Loesener (l. c.), and Léveillé enumerates in his Flore de Kouy-Tchéou the type of *C. salicifolia* under *I. macrocarpa* var. *genuina* Oliv., but does not cite *C. salicifolia* as a synonym.

CELASTRACEAE

Evonymus grandiflora Wallich in Roxburgh, Fl. Ind. ed. Carey, II. 404 (1824).—Loesener & Rehder in Sargent, Pl. Wilson. I. 484

(1913).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 540 (1914).—Léveillé, Cat. Pl. Yun-Nan, 34 (1915).

Evonymus Mairei Léveillé in Fedde, Rep. Spec. Nov. XIII. 260 (1914), excl. specim. "Siao-ho."

CHINA. Yunnan: brousse, pied de mont. à Tong-tchouan, alt. 2500 m., *E. E. Maire,* May 1912 "petit arbre, feuilles caduques, fleurs blanc-jaunâtre"; haies à pied de mont. à Tche-hai, alt. 2500 m., *E. E. Maire,* Aug. 1912 "petit arbre, toujours vert, fleurs jaunâtres, fruits item"; brousse des rochers â Ma-hong, alt. 2700 m., *E. E. Maire,* May 1912 "petit arbre, fleurs jaunes, fruits rouges"; (all three syntypes of *E. Mairei;* photos. in A. A.).

Evonymus Mairei was first identified with *E. grandiflora* by Loesener (l. c.).

Evonymus yunnanensis Franchet in Bull. Soc. Bot. France, XXXIII. 454 (1886).

Evonymus Mairei Léveillé in Fedde, Rep. Spec. Nov. XIII. 260 (1914), quoad specim. "Siao-ho."

CHINA. Kweichou: mont. derrière Siao-ho, alt. 2800 m., *E. E. Maire,* June 1912 "grande arbuste, feuilles caduques, fleurs jaunâtres" (syntype of *E. Mairei;* photo. in A. A.).

The specimen cited above was identified with *E. yunnanensis* by H. F. C[omber] according to a note on the sheet. Specimens recently collected in Kweichou by Y. Tsiang under nos. 9124 and 9133 belong here.

Evonymus uniflora Léveillé & Vaniot in Bull. Soc. Agr. Sci. Arts Sarthe, LIX. 320 (Bouquet Fl. Chine, 5) (1904); in Fedde, Rep. Spec. Nov. VI. 374 (1908); Fl. Kouy-Tchéou, 73 (1913).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 539 (1914).

Evonymus Blinii Léveillé in Fedde, Rep. Spec. Nov. XIII. 259 (1914), quoad specim. Esquirol, no. 478.

CHINA. Kweichou: Pin-fa, sud ouest, bord d'un ruisseau, *J. Cavalerie,* no. 256, Aug. 21, 1902 (holotype of *E. uniflora;* merotype in A. A.); without locality, *J. Esquirol,* no. 478, (fruit) (syntype of *E. Blinii,* and cited in Fl. Kouy-Tchéou; photo. in A. A.).

There are two entirely different specimens marked Esquirol, no. 478, in the Léveillé herbarium. One is a fruiting specimen, named on the label *E. uniflorus* by Léveillé; this is apparently the syntype of *E. Blinii,* upon which the description of the fruit was based. The other is a flowering specimen which bears the name *E. Blinii* in pencil in Léveillé's handwriting, which may be the specimen referred by Loesener to *E. theaefolia* Wall., but I am unable to distinguish this specimen which is in flower, from *E. Forbesiana* Loes.

This species has also been collected recently in Kweichou by Y. Tsiang (no. 4579).

Evonymus theaefolia Wallich, Cat. 4293 (1828), nomen.—M. A. Lawson in Hooker f., Fl. Brit. Ind. I. 612 (1875).—Léveillé, Fl. Kouy-Tchéou, 73 (1914).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 541 (1914).

? *Evonymus Blinii* Léveillé in Fedde, Rep. Spec. Nov. XIII. 259 (1914) quoad specim. "Esquirol, no. 478."

The syntype of *E. Blinii*, J. Esquirol, no. 478 (flowers), was first identified by Loesener (l. c.) with *E. theaefolia* Wall. as "*E. theifolia* var. vel forma" and is cited by Léveillé as a synonym of the latter. The specimen, however, of *E. Blinii*, Esquirol, no. 478, which I have before me, I cannot distinguish from *E. Forbesiana* Loes., but there may be or may have been another specimen of the same number which I have not seen and which may belong to *E. theaefolia* Wall. See also my remarks under the preceding species.

Evonymus theaefolia Wall. occurs in Kweichou, as Y. Tsiang's no. 7274 shows which was collected in 1930 near Gan-wu, Lohou in southern Kweichou.

Evonymus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. XIII. 261 (1914); Fl. Kouy-Tchéou, 71 (1914).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 539 (1914).

CHINA. Kweichou: Tang-tchang (Hoang-tsao-pa), *J. Esquirol*, no. 1569, June 1909 (holotype; merotype in A. A.).

Related to *E. myriantha* Hemsl. according to Loesener from which it differs chiefly in the much smaller leaves and denser less branched cymes.

Evonymus Leclerei Léveillé in Fedde, Rep. Spec. Nov. XIII. 260 (1914); Fl. Kouy-Tchéou, 72 (1914).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 539 (1914).

CHINA. Kweichou: Ma-jo, *J. Cavalerie*, no. 3058, Sept. 5, 1907 (holotype; merotype in A. A.).

This species is like the preceding closely related to *E. myriantha* Hemsl. from which it differs in the longer leaves more densely and distinctly reticulate above.

Evonymus centidens Léveillé in Fedde, Rep. Spec. Nov. XIII. 262 (1914); Cat. Pl. Yun-Nan, 34 (1915).—Loesener, in Ber. Deutsch. Bot. Ges. XXXII. 262 (1914).—Rehder in Jour. Arnold Arb. XI. 164 (1930).

CHINA. Yunnan: collines broussailleuses a Long-ky, alt. 700 m., *E. E. Maire*, June 1912 "grand arbuste à feuilles caduques, fleurs jaunâtres, fruits rouges" (holotype; merotype in A. A.).

Of this species I gave a full description in 1930 (l. c.) based on the specimen cited above and Fang's no. 5819.

Evonymus Dielsiana Loes. var. γ **latifolia** Loesener in Bot. Jahrb. xxx. 455 (1902); in Ber. Deutsch. Bot. Ges. xxxii. 540 (1914).

Evonymus Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. xiii. 259 (1914).

Evonymus Dielsiana Léveillé, Fl. Kouy-Tchéou, 71 (1914), non Loesener sensu stricto.

China. Kweichou: Pin-fa, rochers de Ouen-pi, Li-tseou-gai, *J. Cavalerie,* no. 87, July 23, 1902 "haut 3-4 m." (syntype of *E. Cavaleriei;* merotype in A. A.); Pin-fa, *J. Cavalerie,* no. 865, Feb. 17, 1903 (syntype of *E. Cavaleriei;* photo. in A. A.).

Evonymus Cavaleriei Léveillé in Fedde (not in his Fl. Kouy-Tchéou) was first identified with *E. Dielsiana* var. *latifolia* by Loesener, but Léveillé in his Flore de Kouy-Tchéou omits the variety.

Evonymus Feddei Léveillé in Fedde, Rep. Spec. Nov. xiii. 260 (1914); Fl. Kouy-Tchéou, 72 (1914).—Loesener in Ber. Deutsch. Bot. Ges. xxxii. 539 (1914).

China. Kweichou: route de Pin-fa à Lo-fou, *J. Cavalerie,* no. 3353, April 1908 (holotype; merotype in A. A.).

According to Loesener this species is related to *E. attenuata* Wall. and *E. bullata* Wall.; from the former it differs in the much larger leaves and more branched inflorescences and from the latter in the narrower leaves and less branched inflorescences.

Evonymus Rehderiana Loesener in Sargent, Pl. Wilson. i. 488 (1913); in Ber. Deutsch. Bot. Ges. xxxii. 540 (1914).—Léveillé Fl. Kouy-Tchéou, 73 (1914).

Evonymus bicolor Léveillé in Fedde, Rep. Spec. Nov. xiii. 260 (1914).

Evonymus proteus Léveillé, l. c.

China. Kweichou: Long-ly, Ma-jo, *J. Cavalerie,* no. 2238 (in part), Nov. 13, 1907 "arbre" (holotype of *E. bicolor;* photo. in A. A.); Paitchen, *J. Cavalerie,* no. 2238 (in part), March 30, 1905 "3 or 4 m. de haut, les fleurs et les jeunes feuilles vert-jaunes" (holotype of *E. proteus;* photo. in A. A.).

Evonymus bicolor and *E. proteus* were first identified with *E. Rehderiana* by Loesener (l. c. 1914). Both specimens cited bear the number 2238. The published name *E. proteus* for the specimen from Paitchen does not appear on the original label, but instead another name referring to the color of the branches. Also collected in Kweichou by Y. Tsiang (nos. 5121 and 9029).

Evonymus Forbesiana Loesener in Bot. Jahrb. XXX. 457 (1902); in Ber. Deutsch. Bot. Ges. XXXII. 540 (1914).—Léveillé, Fl. Kouy-Tchéou, 72 (1914).

Evonymus Crosnieri Léveillé in Bull. Soc. Bot. France, LI. p. cxlvi (1904).

Evonymus Blinii Léveillé in Fedde, Rep. Spec. Nov. XIII. 259 (1914), quoad specim. "Esquirol, no. 478."

Evonymus Vanioti Léveillé, l. c. (1914).

CHINA. Kweichou: Pin-fa, bord des ruisseaux, *J. Cavalerie,* no. 1274, June 2, 1902 "fl. rousse" (holotype of *E. Crosnieri;* photo. in A. A.); Pin-fa, *J. Cavalerie,* no. 1272, March 10, 1902 "fl. rousse" (syntype of *E. Vanioti;* photo. in A. A.); moulins de Tong-tchéou, *J. Esquirol,* no. 3236, July 10, 1912 (syntype of *E. Vanioti;* photo. in A. A.); Pin-fa, *J. Cavalerie,* no. 1273, May 5, 1902 (syntype of *E. Blinii;* photo. in A. A.); without precise locality, "coteaux," *J. Esquirol,* no. 478 (flowers), June 1905 (? syntype of *E. Blinii;* photo. in A. A.).

Evonymus Crosnieri, E. Vanioti and one of the syntypes of *E. Blinii* were first referred to *E. Forbesiana* by Loesener (l. c.), and the first two names placed as synonyms under *E. Forbesiana* by Léveillé in his Flore de Kouy-Tchéou. *Evonymus Blinii* is not cited as a synonym, but Esquirol's no. 478, one of the syntypes of that species which, however, partly belongs to *E. uniflora* Lévl., while Cavalerie, no. 1273, which belongs here, is not cited.

Evonymus aculeata Hemsley in Kew Bull. Misc. Inform. 1893, p. 209.—Sprague in Kew Bull. Misc. Inform. 1908, p. 33.

Echinocarpus hederaerhiza Léveillé in Fedde, Rep. Spec. Nov. x. 474 (1912).—**Synon. nov.**

Evonymus acanthocarpa Léveillé, Fl. Kouy-Tchéou, 71 (1914); non Franchet.

CHINA. Kweichou: Pin-fa, rampant sur les rochers, *J. Cavalerie,* no. 2761, April 1906 (holotype of *Echinocarpus hederaerhiza;* photo. in A. A.).

Echinocarpus hederaerhiza was referred by Léveillé in his Flore de Kouy-Tchéou to *Evonymus acanthocarpa* Franch., but it differs from that species chiefly in the longer compressed spines of the fruit, in the black bud-scales persisting at the base of the branchlets for some time and in the non-verruculose branchlets.

Evonymus acanthocarpa Franchet, Pl. Delavay. 129 (1889).—Sprague in Kew Bull. Misc. Inform. 1908, p. 32.

Echinocarpus erythrocarpa Léveillé in Fedde, Rep. Spec. Nov. x. 474 (1912).—**Synon. nov.**

Evonymus erythrocarpa Léveillé, Fl. Kouy-Tchéou, 72 (1914).

CHINA. Kweichou: environs de Gan-pin, dans la montagne,

L. Martin in hb. Bodinier, no. 2493, Oct. 9, 1898 "arbuste lianeux" (holotype of *Echinocarpus erythrocarpa;* merotype in A. A.).

Evonymus subtrinervis, nom. nov.

Echinocarpus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. x. 474 (1912); non *Evonymus Esquirolii* Léveillé.—**Synon. nov.**

Echinocarpus Cavaleriei Léveillé, l. c.—**Synon. nov.**

Evonymus Blinii Léveillé, Fl. Kouy-Tchéou, 71 (1914); non Léveillé in Fedde, Rep. Spec. Nov. XIII. 259 (1914).

Evonymus Cavaleriei Léveillé, Fl. Kouy-Tchéou, 71 (1914); non Léveillé in Fedde, Rep. Spec. Nov. XIII. 259 (1914).

Frutex glaber, ramis gracilibus tenuiter verruculosis quadrangulatis angulis subalatis; gemmae terminales parvae pauciperulatae, obtusae, pallide cinereo-virescentes. Folia persistentia chartacea, ovato-oblonga vel oblongo-lanceolata, 4.5-9 cm. longa et 1.8-3 cm. lata, acuminata, basi late cuneata vel rarius fere rotundata, fere ad basin denticulata denticulis mucrone fusco inflexo, basi trinervia, costa medio utrinque elevata, nervis lateralibus 4-6, pari inferiore superioribus aequilongo vel longiore, supra in sicco manifeste, subtus leviter elevatis, supra laete viridia, reti nervulorum in sicco visibili, subtus pallida, reti obsoleto; petioli 2-4 mm. longi, tenues. Flores desiderantur. Inflorescentiae fructiferae bis dichotomae; pedunculus circiter 1.5 cm. longus, gracilis, quadrangularis, ramulis circiter 1 cm., pedicellis 2-3 mm. longis; capsula subglobosa, aculeis inclusis circiter 1 cm. diam., pallide flavo-carnea, aculeis complanatis 1-1.5 mm. longis, dissitis non dense aggregatis; semina 6 mm. longa, nigra, arillo pallide aurantiaco apice laciniato aperto excepto inclusa.

China. Kweichou: without precise locality, *J. Esquirol,* no. 844 (holotype of *Echinocarpus Esquirolii, E. Blinii* and *E. subtrinervis;* photo. and merotype in A. A.); without precise locality, *J. Esquirol* (holotype of *Echinocarpus Cavaleriei;* photo. in A. A.).

The specimens cited above I have not been able to identify with any previously described species; they seem nearest to *E. echinata* Wall. from which they are easily distinguished by the leaves being 3-nerved at the base, with fewer veins diverging at a more acute angle. From *E. Hemsleyana* Loes. they differ in the shape of the leaves, shorter petioles, and the shorter spines of the fruit and smaller winter-buds. As Léveillé's description is insufficient I have drawn up a more complete description based on Esquirol no. 844. The other specimen, the type of *Echinocarpus Cavaleriei,* is rather poor with all the leaves dropped from the branches and very young fruit; it differs from the type in the broader leaves, ovate-oblong, partly rounded at base, about 5 cm. long and 2.2 cm. broad.

Unfortunately none of the names proposed by Léveillé can be used

for this species. The two specific epithets under *Echinocarpus* cannot be transferred to *Evonymus* since the resulting combinations are preoccupied and *Evonymus Blinii,* based on *Echinocarpus Esquirolii* is invalidated by an earlier homonym, which by Léveillé was considered non-valid, because it had been reduced to synonymy. It should be noted here that in 1914 three important publications relating to *Evonymus* came out: Léveillé's descriptions of many new species in Fedde's Repertorium on May 5, 1914, Loesener's corrections in Ber. Deutsch. Bot. Ges. on July 30, 1914, and Léveillé's Flore de Kouy-Tchéou which contains these corrections, in September or October of that year.

Evonymus Maackii Ruprecht in Bull. Phys. Math. Acad. Sci. St. Pétersb. XV. 358 (1857).—Loesener in Ber. Deutsch. Bot. Ges. XXX. 540 (1914).—Léveillé, Fl. Kouy-Tchéou, 71 (1914).

Evonymus coreanus Léveillé in Fedde, Rep. Spec. Nov. VIII. 284 (1910).

KOREA: in dumosis Chinnampo, *U. Faurie,* no. 520, Aug. 1906 (holotype of *E. coreanus,* isotype in A. A.).

Evonymus coreanus was first referred to *E. Maackii* by Loesener (l. c.).

Evonymus Hamiltoniana Wallich in Roxburgh, Fl. Ind. ed. Carey, II. 403 (1824).—Loesener in Ber. Deutsch. Bot. Ges. XXX. 541 (1914).—Léveillé, Fl. Kouy-Tchéou, 72 (1914).

Evonymus rugosa Léveillé in Fedde, Rep. Spec. Nov. XIII. 261 (1914).

Evonymus Darrisii Léveillé, l. c.—**Synon. nov.**

Evonymus Maackii Léveillé, Fl. Kouy-Tchéou 73 (1914); non Ruprecht.

CHINA. K w e i c h o u : Hoang-tsao-po, colline de la pagode, *J. Esquirol,* no. 1532 (holotype of *E. rugosa;* photo. in A. A.); without precise locality, *J. Esquirol,* no. 711 (holotype of *E. Darrisii;* photo. in A. A.).

Evonymus rugosa was first referred to *E. Hamiltoniana* by Loesener with some doubt, while he identified *E. Darrisii* with *E. Maackii.* Though *E. Darrisii* in its leaves resembles *E. Maackii* rather than *E. Hamiltoniana,* the fruit seems to agree better with that of the latter species. Also for geographical reasons it seems unlikely that the Kweichou plant belongs to *E. Maackii* which has not yet been recorded from central and western China.

Evonymus lanceifolia Loesener in Bot. Jahrb. XXX. 462 (1902); in Ber. Deutsch. Bot. Ges. XXXII. 541 (1914).—Léveillé, Fl. Kouy-Tchéou, 72 (1914).

Evonymus Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XIII. 261 (1914).

China. Kweichou: Gan-chouen, *J. Cavalerie*, no. 3824, June 1910 "petit arbre" (holotype of *E. Bodinieri;* photo. in A. A.).

Evonymus Bodinieri was referred to *E. lanceifolia* by Loesener with the remark that it approaches *E. Hamiltoniana*. The type specimen consists of two branches, a flowering and a fruiting branch; according to the dark colored anthers the specimen belongs to *E. lanceifolia*, for *E. Hamiltoniana* differs from both closely related species *E. Maackii* and *E. lanceifolia* in the yellowish white anthers. The label of the type specimen bears in Léveillé's handwriting an unpublished name referring to the color of the leaves, to which is added rather indistinctly written in pencil "vel Bodinieri."

Evonymus alata (Thbg.) Regel, Fl. Ussur. 40, t. 7 (1861).—Loesener & Rehder in Sargent, Pl. Wilson. I. 493 (1913).

Microrhamnus Taquetii Léveillé in Fedde, Rep. Spec. Nov. VIII. 284 (1910).—**Synon. nov.**

Korea. Quelpaert: secus torrentes, *T. Taquet*, no. 153, Sept. 1907 (holotype of *Microrhamnus Taquetii;* fragments of type and isotype in A. A.).

Besides the type specimen the cover of *Microrhamnus Taquetii* contains Taquet, no. 4095, consisting of flowering branches of *E. alata;* both specimens belong to the form *aptera* Regel with the branches not or very slightly winged.

Evonymus disticha Léveillé in Fedde, Rep. Spec. Nov. XIII. 261 (1914); Fl. Kouy-Tchéou, 71 (1914).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 540 (1914).

China. Kweichou: environs de Kouy-yang, bois de la pagode de Kien-lin-chan, *E. Bodinier*, no. 2455, June 27, 1898 "petit arbuste, fleurs jaunes" (holotype, merotype in A. A.).

This species was collected also at the same locality in 1930 by Y. Tsiang, no. 8488. This specimen bears flowers and young fruits which differ from those of *E. alata* in their shorter and broader lobes.

Celastrus Vanioti (Lévl.), comb. nov.

Saurauja Vanioti Léveillé, Fl. Kouy-Tchéou, 415 (1915, before October.)

Celastrus spiciformis Rehder & Wilson in Sargent, Pl. Wilson. II. 348 (1915, Dec. 28).—**Synon. nov.**

China. Kweichou: environs de Kouy-yang, route du Collège, dans les haies, *E. Bodinier*, May 30, 1898 "liane ligneuse" (holotype of *Saurauja Vanioti;* photo. and merotype in A. A.).

Saurauja Vanioti is without doubt identical with *Celastrus spiciformis*, the former differing only slightly in the somewhat more reticulate leaves resembling in this respect *C. gemmata* Loes., but paler on the under

surfaces. As *Saurauja Vanioti* was published several months earlier, the Flore de Kouy-Tchéou having been received at the Arnold Arboretum in October 1915, the transfer of the specific epithet to *Celastrus* becomes necessary.

Celastrus gemmata Loesener in Bot. Jahrb. XXX. 468 (1902).—Rehder & Wilson in Sargent, Pl. Wilson. II. 352, (1915).

Embelia Esquirolii Léveillé in Fedde, Rep. Spec. Nov. x. 374 (1912).—**Synon. nov.**

CHINA. K w e i c h o u : Collège, *J. Esquirol*, no. 4, May 10, 1906 "arbre, fleurs blanc-verdâtres" (holotype of *Embelia Esquirolii*, merotype in A. A.).

Celastrus gemmata has been collected in Kweichou by Y. Tsiang under nos. 4661, 5360 and 6450.

Celastrus stylosa Wallich in Roxburgh, Fl. Ind. ed. Carey, II. 401 (1924).—Léveillé, Fl. Kouy-Tchéou, 69 (1914).

Celastrus Cavaleriei Léveillé in Monde Pl. sér. 2, XVIII. 31 (1916); non Léveillé (1914).

CHINA. K w e i c h o u : Pin-fa, ruisseaux, *J. Cavalerie*, no. 496, Sept. 16, 1902 (holotype of *C. Cavaleriei;* photo. in A. A.).

Celastrus Cavaleriei was by Loesener according to his note on the type sheet referred to *C. stylosa* or a related species.

Celastrus flagellaris Ruprecht in Bull. Acad. Sci. St. Pétersb. sér. 3, XV. 357 (1857); Decas Pl. Amur. t. 4 (1859).—Loesener in Ber. Deutsch. Bot. Ges. XXX. 541 (1914).—Rehder & Wilson in Sargent, Pl. Wilson. II. 357 (1915).

Celastrus clemacanthus Léveillé in Fedde, Rep. Spec. Nov. VIII. 284 (1910).

KOREA. Q u e l p a e r t : scandens in muris agrorum Haouen, *T. Taquet*, no. 632, May 8, 1908 (holotype of *C. clemacanthus;* photo. and isotype in A. A.).

Celastrus clemacanthus Léveillé was identified with *C. flagellaris* by Loesener (l. c.).

Celastrus Hindsii Benth. var. **Henryi** Loesener in Bot. Jahrb. XXIX. 444 (1900); XXX. 467 (1902).

Erythrospermum Cavaleriei Léveillé, Fl. Kouy-Tchéou, 51 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : Gan-chouen, rochers, *J. Cavalerie*, no. 3976, in 1912, "liane" (holotype of *Erythrospermum Cavaleriei;* merotype in A. A.).

Gymnosporia Esquirolii Léveillé in China Rev. Ann. 1916, p. 18 (1916).—Schneider in Oester. Bot. Zeitschr. LXVII. 140, in nota (1918).

Berberis Cavaleriei Léveillé, Fl. Kouy-Tchéou, 48 (1914); non Léveillé in Fedde, Rep. Spec. Nov. IX. 454 (1911).

Berberis Esquirolii Léveillé, Fl. Kouy-Tchéou, 47, in clavi (1914).

China. Kweichou: Lo-fou, colline du fort, 800 m., terrain rocheux aride, *J. Esquirol,* no. 3645, June 20, 1912 (holotype of *Berberis Cavaleriei;* merotype in A. A.).

Gymnosporia Esquirolii is closely related to *G. diversifolia* Maxim. but easily distinguished by the slender peduncles of the inflorescence 0.5-1.5 cm. long and by the closely and finely crenulate or serrulate leaves, often acute at the apex and of thinner texture. To this species I also refer Siméon Ten, nos. 169 and 378, from Pe-yen-tsin, Henry, no. 9391, from Mengtze, and Forrest no. 10738. *Gymnosporia Esquirolii* seems to be restricted to Yunnan and Kweichou, while *G. diversifolia* Maxim. is widely distributed through southeastern China, Indochina, Formosa and the Liukiu Islands.

Gymnosporia Esquirolii was published without reference to a specimen, only with the citation of *Berberis Cavaleriei* as a synonym. This citation refers without doubt to *B. Cavaleriei* Léveillé, Fl. Kouy-Tchéou, 48, which appears in the key on the preceding page as *B. Esquirolii.* This species is based on Esquirol's no. 3645 and fragments were sent to C. Schneider by Léveillé in November 1915 as *B. Esquirolii,* but without indication of collector and number. These fragments agree with the specimen cited above and were referred by Schneider to *Gymnosporia* (in Sargent, Pl. Wilson. II. 359). Léveillé apparently had first given to Esquirol's no. 3645 the name *B. Esquirolii,* but when Schneider, to whom Léveillé had sent in October 1913 specimens of his earlier *B. Cavaleriei* based on Cavalerie, no. 3209, and published in 1911 (in Fedde Rep. Spec. Nov. IX. 454), had referred this species to *B. Griffithiana* Wall., he changed the name of his not yet published *B. Esquirolii* to *B. Cavaleriei,* wishing to keep Cavalerie's name attached to a valid species, but forgot to change the name in the key. *Berberis Cavaleriei* of 1911, however, which was in 1913 considered a synonym of *B. Griffithiana* by Schneider, and was accepted as such by Léveillé in his Flore de Kouy-Tchéou with the citation of "*B. Cavaleriei* Lévl. olim" as a synonym, was revived as a valid species by Schneider in 1918 (in Oesterr. Bot. Zeitschr. LXVII. 140) and applied to the Chinese species formerly referred to *B. Griffithiana* which according to his revised opinion does not occur at all in China.

Gymnosporia acuminata Hooker f., Fl. Brit. Ind. I. 619 (1875).

Evonymus yunnanensis Léveillé, Fl. Kouy-Tchéou, 73 (1914); non Franchet.

China. Kweichou: Lo-fou, *J. Cavalerie,* no. 3530, March 1909 (cited under *E. yunnanensis* Franch. by Léveillé; photo. in A. A.).

The specimen cited above differs from typical *G. acuminata* of which

I have isotypes of Hooker's and Griffith's specimens before me, in the smaller and narrower scarcely acuminate leaves 7-9 cm. long and 2-3 cm. broad and rather closely serrulate to near the base, but fruit and inflorescence does not seem to differ. It looks rather different and I was inclined to consider it at least a distinct variety, if not Hemsley's no. 13437 from Szemao represented an intermediate form, agreeing in the size of the leaves with the type and in the serration, though less dense and fine, with Cavalerie's specimen.

Tripterygium hypoglaucum (Lévl.) Hutchinson in Kew Bull. Misc. Inform. 1917, p. 101.—Léveillé, China Rev. Ann. 1916, p. 23 (Mscr.).

Aspidopterys hypoglauca Léveillé in Fedde, Rep. Spec. Nov. IX. 458 (1911).

Pentace Virginis Léveillé in Monde Pl. sér. 2, XVIII. 28 (1916).—**Synon. nov.**

CHINA. K w e i c h o u : Ma-jo, *J. Cavalerie*, no. 3316, Aug. 1908 "petit arbre lianeux" (holotype of *Aspidopterys hypoglauca;* merotype in A. A.). Y u n n a n : brousse du plateau de Ie-ma-tchouan, 3300 m., *E. E. Maire*, June 1912 "arbuste grimpant, long rameaux, fleurs blanches, fruit rouge sombre" (holotype of *Pentace Virginis;* photo. in A. A.).

Hutchinson first recognized *Aspidopterys hypoglauca* as a *Tripterygium* and communicated the resulting new combination to Léveillé who published it in his "China; revue annuelle 1916" distributed as manuscript to the larger botanical institutions before Hutchinson's paper was printed.

(*To be continued*)

HERBARIUM, ARNOLD ARBORETUM,
HARVARD UNIVERSITY.

VARIATION IN FLOWER COLOR IN HAMAMELIS VERNALIS

EDGAR ANDERSON

With one text figure

WHATEVER the ultimate explanation, there seem to be certain species of plants in which the variation between one individual and another is unusually marked. *Hamamelis vernalis* is such a species, for the plants of a single locality vary markedly in flower color, color pattern, time of blooming, and the number and arrangement of the flowers on the flowering branches. In this respect the species stands in sharp contrast to *H. virginiana* which, though it runs into geographical varieties, is comparatively uniform in any one locality. In *Hamamelis virginiana*, variation in flower color, while not unknown, is extremely rare. Examination of many individuals in eastern Massachusetts has revealed only two with a faint flush of red at the base of the petals. At Sterling, Massachusetts, for instance, of 35 individuals examined, 34 were pure yellow and one was flushed with red on the calyx and at the base of the petals. There is at the Arnold Arboretum a bush presumably collected in eastern Massachusetts which has light red petals and a yellowish green calyx (Rehder, 1922). A similar bush was collected some years ago near Malden, Massachusetts, by Mr. Edward L. Rand (Sargent, 1893). Mr. O. A. Farwell has collected a similar form with red calyx lobes at Stony Creek, Michigan and it has been distributed as Farwell, no. 3943. Variation in flower color is therefore not unknown in *H. virginiana*, though it is comparatively infrequent.

In *H. vernalis* nearly every bush has its own distinctive flower color and it is not at all uncommon to find pure yellow-flowered plants growing side by side with red-flowered ones. These differences seem to be germinal, since there is little variation between the flowers on a single bush and since the peculiarities of a particular bush persist after transplantation. The specimens in the living collections of the Arnold Arboretum maintain their characteristic flower colors season after season and the colors are perpetuated in plants propagated vegetatively. One color form has already received taxonomic recognition, *Hamamelis vernalis* forma *carnea* Rehd.

The following records of variation in natural populations of *H. vernalis* (Tables 1 & 2) were made in St. Francis County, Missouri, on February 28, 1931. In each case the bushes, as is usual in this species, were growing in gravelly creek beds. At each locality, for a distance of

approximately one-eighth of a mile along the watercourse, a single representative twig was taken from each bush then in flower. The specimens were then taken to the laboratory and a record was made of their color and color pattern. The underlying yellow pigment seems to be practically the same shade throughout all the plants and nearly all of the variation in color is due to variations in the intensity and distribution of the red pigment, which was found to be a water soluble substance occurring in the epidermis of the petals and sepals. In the petals it varies from none at all, to a faint flush at the base, through intermediate stages up to 85% of the length. In their general tone the flowers varied from Light Cadmium Yellow (Ridgway) to Dragon's Blood Red (Ridgway). The coloring always extends up from the base of the petals and even the reddest flowers were yellow at the very tips of the petals. On the sepals the color seems to spread from the mid-vein. In the following records it has been summarized under four grades:

"pure yellow."	no red pigment in the sepal.
"line plus."	sepal red along the mid-vein with a faint flush of red at either side.
"all but margin."	sepal red with a narrow yellow margin at either side.
"entire."	red pigment distributed over the entire sepal.

It will be seen that while the variation at the two localities was similar, that the colors were on the average a little darker at Flat River than at Libertyville. While the intensity of the red coloring on the petals was associated with the coloring of the sepal, the correlation was not perfect. This is shown graphically in Table 2 which summarizes the data from both localities.

The above tables present a graphic and objective summary of variation in color pattern. The size and development of the flowering branches seem to be quite as variable, though it is difficult to record the variation objectively. My colleague, Mr. Ernest J. Palmer, tells me that there is a correspondingly great variation in the degree of pubescence of the leaves. This has been given varietal recognition in *H. vernalis* var. *tomentella* (Rehd.) E. J. Palmer.

It is particularly interesting to find such variation in *H. vernalis* since it is a species of very limited distribution, being known only from the Ozark Mountains and adjacent lowlands to the south and west. It may well be an ancient species like many others in that area (Palmer and Steyermark, in press), since its closest affinities are with the Asiatic species of *Hamamelis* rather than with the American *H. virginiana*. Like them it is winter-flowering, like them it has an extensive develop-

ment of red pigment in the flower, like some of them it has a tendency to pubescent leaves. The genus is known from its fossil record (Berry, 1916) to be an ancient one which was formerly wide-spread in the northern hemisphere.

It is commonly said that species of limited distribution are less variable than those more widely distributed. As far as *Hamamelis vernalis* and *H. virginiana* are concerned the reverse seems to be true. The approximate ranges of the two species are shown in Figure 1. *Hamamelis virginiana* covers an area many times as large as that of *H. vernalis* and yet in any one locality the variation in color, habit, and pubescence is much less. It is of course quite possible that variability

TABLE 1. Variation in petal color and sepal color of 63 plants of *Hamamelis vernalis* examined at Libertyville and Flat River, Missouri. Further explanation in the text.

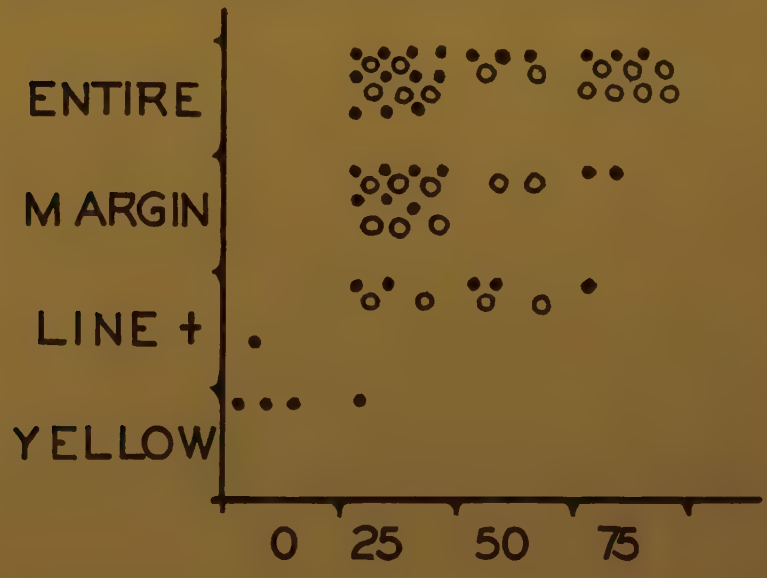
Libertyville, Missouri

% of petal red	distribution of red in calyx	% of petal red	distribution of red in calyx
0	pure yellow, flush of red at petal base	50	line plus
25	line plus	50	entire, faint
75	entire	25	entire, faint
25	entire, faint	25	entire
50	entire, faint	25	entire
25	all but margin	75	all but margin
0	line plus	25	entire, faint
25	entire, faint	25	all but margin
50	entire, dark	25	entire
25	entire, faint	25	pure yellow
25	entire	25	entire
0	pure yellow, pure lemon yellow	25	entire
75	entire	25	all but margin, faint
75	line plus	25	all but margin, faint
0	faint flush, flush at petal base	50	line plus
75	entire	75	all but margin
25	line plus	25	all but margin
25	all but margin		

TABLE I (Continued)
Flat River, Missouri

% of petal red	distribution of red in calyx	% of petal red	distribution of red in calyx
50	line plus	25	all but margin
25	entire	75	dark, all but margin
25	line plus	50	all but margin
25	all but margin	25	all but margin
75	entire	75	entire
25	all but margin	50	entire
25	line plus	50	entire
25	entire	75	entire
25	entire, dark	75	entire, dark
50	all but margin	75	entire
25	entire, faint	75	entire
25	entire, faint	75	entire
25	all but margin	50	line plus
25	all but margin		

TABLE 2. Correlation in petal color (vertical scale) and sepal color (horizontal scale). Each dot represents the combination found in a single individual. Solid dots, Libertyville; open circles, Flat River.



between plants in any one locality and variation between one region and another are quite different processes. As far as *inter*-regional variation is concerned *H. virginiana* is perhaps quite as variable as *H. vernalis*.

FIGURE 1. Approximate distribution of *Hamamelis vernalis* (solid black) and *Hamamelis virginiana* (diagonal lines).

SUMMARY

Hamamelis vernalis, a species of very limited distribution, is shown to exhibit a high degree of *intra*-regional variability.

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ARNOLD ARBORETUM,
HARVARD UNIVERSITY.

NOTES ON THE GENUS PINUS

THE BLACK CONE OF PINUS PONDEROSA

GEORGE RUSSELL SHAW

IN SARGENT'S SILVA and in his Manual, also in Sudworth's Forest Trees of the Pacific Slope and in his Pine Trees of the Rocky Mountain Region, some of the cones of *Pinus ponderosa* Douglas are described as dark purple (nearly black) in color. The quotation below is copied from Sudworth in Bull. U. S. Dept. Agric. No. 460, p. 31 (1917).

"The cones of some trees are a bright grass-green when mature, while those of other trees are a dark purple, there being no essential difference between trees bearing cones so dissimilar in color."

In autumn when the cones are completely dried, the purple color disappears and is never found in herbarium collections. The ultimate color is brown, never purple or black. The explanation is simple. The purple color is a transient intermediate stage between the summer-green and the autumn-brown coloration of the maturing cones. Its life is brief and, as a consequence, it has escaped the notice of collectors. I have happened on it in five or six species, such as *Pinus rigida* Miller where the intermediate color is brown and the autumn-color is yellow, and also in *Pinus Greggii* Engelmann, where the intermediate color, strange to say, is a brilliant scarlet and the autumn-color is Naples yellow. The purpose of this article is to call attention to the intermediate color and to prevent further error from that source.

THE CAMBIUM AND ITS DERIVATIVE TISSUES NO. VIII. STRUCTURE, DISTRIBUTION, AND DIAGNOSTIC SIGNIFICANCE OF VESTURED PITS IN DICOTYLEDONS

I. W. Bailey

With four text figures and plates 61-63

INTRODUCTION

In connection with an extended investigation of plasmodesma, I have had occasion to examine the cell walls and the pit membranes of a wide range of Gymnosperms and Angiosperms. It is evident that many of the structures in the tissues of the higher plants which are hypothesized as evidence for the existence of protoplasmic connections can not be interpreted as such.

The bordered pits in the vessels of the Leguminosæ and of certain other families of Dicotyledons are referred to as "sievelike" or "cribriform," a nomenclature based upon the assumption that the pit membranes are perforated by numerous small openings through which protoplasmic connections occurred in the immature vessel members. The sievelike appearance described by Dutailly (1), Jönsson (4), and others is not due to perforations of the pit membrane, but, as will be shown on the following pages, to minute outgrowths from the free surface of the secondary walls.

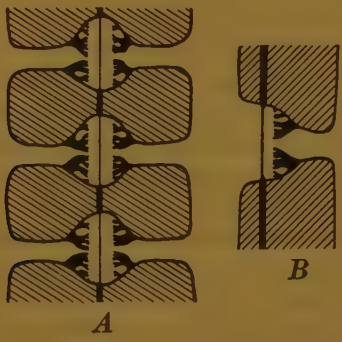
Although the so-called cribriform pits of tracheary tissue lose much of their putative physiological significance, they appear to be of considerable value both in the systematic study of woods and in discussions concerning the relationships and classification of specific groups of Dicotyledons.

STRUCTURE OF VESTURED (CRIBRIFORM) PITS

In unstained longitudinal sections of the wood of the Leguminosæ, Myrtaceæ, Polygonaceæ, Lythraceæ, Combretaceæ, and of a number of other representatives of the Dicotyledons, the bordered pits have, in surface view, a punctate appearance due to the presence of refractive processes of varying forms. In sections treated differentially with Haidenhain's hæmatoxylin and safranin, these processes are deeply stained and, in photomicrographs, appear as dark spots (*Plate 61, figs. 1, 3, 5,* and *7*) or as reticulate structures (*Figs. 2, 4,* and *6*) on a lighter colored background. By carefully focusing at successive levels it is

possible to demonstrate that there are two entirely independent sets of deeply-staining processes in each bordered pit-pair and that the punctate appearance of the bordered pits in surface view is not due to an unevenly thickened or perforated pit membrane.

Owing to the small size of the pits and to the thickness of the walls in the tracheary elements of most Dicotyledons, the internal structural details of bordered and half-bordered pit-pairs may be observed most accurately in sectional rather than in surface views. It should be emphasized in this connection, however, that for this purpose extremely thin sections, 5-7 microns, are essential. The pits illustrated in *Plate 61, fig. 7* are shown in section in *Plate 62, fig. 10*. The thick, imperforate pit-membranes are in the median position, and the pit apertures and pit chambers are clearly visible. The dark-colored, toothlike processes obviously are attached to the overarching walls of the pit chambers and are not connected with the pit membranes. The more massive papillæ

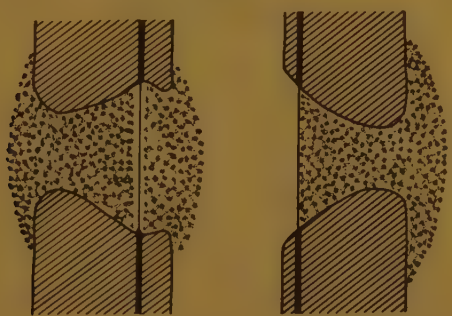


TEXT FIGURE 1. COMBRETUM SPECIES. (A) Sectional view of bordered pit-pairs in the walls of adjacent vessels, showing coralloid outgrowths from the overarching walls of the pit-chambers. (B) Sectional view of half-bordered pit-pair in the adjacent walls of a vessel (right) and of a parenchymatous element (left). The bordered pit is vestured, but the simple pit is not.

are attached close to the margins of the pit apertures and project diagonally towards the center of the pit chambers. Thus, in *Plate 61, fig. 7*, they are visible through the pit apertures, whereas the smaller peripheral papillæ, on the contrary, are partly obscured by the intervening portion of the secondary wall. *Plate 62, fig. 8* and *Text fig. 1A* are sections through the bordered pits illustrated in *Plate 61, fig. 5*. In these pits, as in the preceding ones, the deeply-stained processes are attached to the overarching walls and project into the pit chambers. They are characterized by having a distinctly branched or coralloid

structure, however. The pit membranes are shown in the median position in *Text fig. 1*, whereas in *Plate 62, fig. 8* they are rumpled and deflected to the right or left. A somewhat different type of structure is shown in *Plate 62, fig. 11*, a section through the bordered pits illustrated in *Plate 61, fig. 4*. Here the dark-colored processes form loose mats of branching and anastomosing filaments which are attached to the overarching walls of the pit chambers. Denser mats of finer texture which occlude the pit chambers are illustrated in *Plate 61, fig. 2* and *Plate 63, fig. 17*.

TEXT FIGURE 2. EUGENIA DICHOTOMA DC. Sectional view of bordered pit-pair in the walls of adjacent fiber-tracheids, showing papillary projections from the margins of both the inner and the outer apertures.



TEXT FIGURE 3. PARASHOREA PLICATA Brandis. (A) Sectional view of bordered pit-pair in the adjacent walls of a vessel (left) and of a short tracheid (right). Mats of fine texture fill the entire pit-cavities and project more or less into the lumens of the cells. (B) Sectional view of half-bordered pit-pair in the adjacent walls of a vessel (right) and of a parenchymatous element (left). The bordered pit is vestured, but the simple pit is not.

The papillary, coralloid or filamentous processes are not confined to the pit chambers in all cases. As shown in *Plate 62, fig. 12* and *Text fig. 2*, they may be attached to the margins of both the inner and the outer apertures. They may fill the entire pit cavities and project more

or less into the lumens of the cells (*Plate 62, fig. 9* and *Text fig. 3A*). Not infrequently they occur on the inner surface of the secondary walls of the vessels (*Text fig. 4* and *Plate 63, fig. 16*) as well as within the bordered pits. They appear to be confined to tracheary elements, however. Thus, in half-bordered pit-pairs, they are present in the bordered pits of the tracheary elements but are absent in the simple pits of the adjoining parenchymatous cells (*Plate 62, fig. 13* and *Plate 63, fig. 18, Text figs. 1B* and *3B*).

TEXT FIGURE 4. VOCHYSIA HONDURENSIS Sprague. Sectional view of bordered pit-pairs in the walls of adjacent vessels, showing branched and anastomosing projections from the overarching walls of the pit-chambers and from the inner surfaces of the vessels.

In view of such facts as these, the terms *sievelike* or *cribriform* obviously should not be used in discussing the structures originally described by Dutailly and Jönsson. The terms *vestured pits* and *vestured walls* have been substituted for them by the Nomenclature Committee of the International Association of Wood Anatomists.

DEVELOPMENT OF VESTURED PITS

A priori, the structure of vestured pits and of vestured walls might be interpreted as due to adhering extraneous material deposited in mature tracheary cells during post mortem changes in the drying of sapwood or during the transformation of sapwood into heartwood. A study of living cells in sections of differentiating xylem reveals the fact that the curious processes in reality are formed by the cytoplasm during the later stages of the development of tracheary elements.

It should be noted in this connection, however, that punctate appearances (*Plate 63, fig. 15*) may be produced at times by extraneous or coagulated material which accumulates in the bordered pits during post

mortem changes and particularly during the transformation of sapwood into heartwood. True vestured pits may be distinguished from such artifacts by constancy of form and distribution, as well as by differential solubilities and other tests. In all the plants examined by me the vestured intervascular pits, when present in a species, occur in all vessels throughout a given specimen, in specimens from different parts of a tree, and in material from widely separated sources. The artifacts, on the contrary, are of extremely irregular and sporadic occurrence, not only in different specimens of a particular species, but even within the limits of a single section.

DISTRIBUTION OF VESTURED PITS IN DICOTYLEDONS

According to Record (6), pits with so-called cribriform membranes have been reported by Jönssen (4), Heiden (2), Ursprung (7), Moll and Janssonius (5), and Record as occurring in the secondary xylem of 20 families of Dicotyledons. In most cases, the number of species and genera listed is so restricted that it is not possible to formulate reliable conclusions concerning the occurrence and diagnostic value of vestured pits in specific groups of Dicotyledons. In 11 of the 20 families, the character is recorded in a single species or genus.

It seems advisable, accordingly, to tabulate my observations upon 2660 species, 979 genera, 152 families, and 33 orders. Families with vestured pits are printed in italics in Table 1. The numbers of genera and species investigated in each family are recorded in the columns at the right. Families in which "cribriform pits" have been reported by other investigators are marked with an asterisk.

TABLE I.

Orders and Families	Number of genera studied	Number of species studied
VERTICILLATAE		
Casuarinaceæ	1	8
PIPERALES		
Piperaceæ	2	3
Chloranthaceæ	1	1
SALICALES		
Salicaceæ	2	33
GARRYALES		
Garryaceæ	1	1
MYRICALES		
Myricaceæ	2	7

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
LEITNERIALES		
Leitneriaceæ	1	1
JUGLANDALES		
Juglandaceæ	5	24
FAGALES		
Betulaceæ	5	48
Fagaceæ*	7	107
URTICALES		
Ulmaceæ	6	33
Moraceæ	15	52
Urticaceæ	10	27
PROTEALES		
Proteaceæ	26	135
SANTALALES		
Santalaceæ	2	5
Olacaceæ	5	6
Octoknemataceæ	1	1
Loranthaceæ	2	2
ARISTOLOCHIALES		
Aristolochiaceæ	1	1
POLYGONALES		
*Polygonaceæ**	8	48
CENTROSPERMAE		
Chenopodiaceæ	5	8
Amarantaceæ	1	1
Nyctaginaceæ	2	6
Aizoaceæ	1	1
Caryophyllaceæ	1	1
RANALES		
Trochodendraceæ	3	4
Himantandraceæ	1	1
Cercidiphyllaceæ	1	1
Ranunculaceæ	4	4
Lardizabalaceæ	1	1
Berberidaceæ	1	3
Menispermaceæ	1	1
Magnoliaceæ	10	33
Annonaceæ	16	31

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
Myristicaceæ	4	6
Monimiaceæ	3	3
Lauraceæ*	19	47
Hernandiaceæ*	4	6
RHOEADALES		
Papaveraceæ	3	4
Capparidaceæ	*8*	*28*
Cruciferæ	*3*	*4*
Moringaceæ	1	2
ROSALES		
Saxifragaceæ	7	9
Pittosporaceæ	3	17
Brunelliaceæ	1	1
Cunoniaceæ	3	6
Hamamelidaceæ*	8	9
Eucommiaceæ	1	1
Platanaceæ	1	4
Crossosomataceæ	1	1
Rosaceæ*	24	77
Connaraceæ	4	8
*Leguminosæ**	*91*	*198*
Bauhinieæ	2	19
GERANIALES		
Oxalidaceæ	1	1
Linaceæ	1	1
Humiriaceæ	2	2
Erythroxylaceæ	1	4
Zygophyllaceæ	3	7
Rutaceæ	22	46
Simarubaceæ	9	16
Burseraceæ	7	15
Meliaceæ	18	56
Malpighiaceæ	*13*	*33*
*Vochysiaceæ**	*3*	*12*
Trigoniaceæ	1	5
Polygalaceæ	4	4
Dichapetalaceæ	1	1
Euphorbiaceæ*	35	55
Brideliæ	*2*	*6*

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
SAPINDALES		
Buxaceæ	1	4
Coriariaceæ	1	1
Anacardiaceæ	20	61
Cyrillaceæ	2	2
Aquifoliaceæ	3	12
Celastraceæ	23	53
Salvadoraceæ	1	1
Staphyleaceæ	2	4
Icacinaceæ	6	7
Aceraceæ	1	25
Hippocastanaceæ*	1	6
Sapindaceæ	26	48
Sabiaceæ	2	3
RHAMNALES		
Rhamnaceæ*	9	24
Vitaceæ	3	7
MALVALES		
Elaeocarpaceæ	4	15
Tiliaceæ	12	24
Malvaceæ	9	11
Bombacaceæ	5	8
Sterculiaceæ	9	31
Scytopetalaceæ	1	1
PARIETALES		
Dilleniaceæ	4	14
Eucryphiaceæ	1	3
Ochnaceæ-Exalbuminosæ	*5*	*21*
Albuminosæ	2	4
Caryocaraceæ	1	1
Marcgraviaceæ	1	2
Theaceæ	8	11
Guttiferæ*	17	25
Dipterocarpaceæ	*8*	*32*
Fouquieraceæ	1	1
Cistaceæ	1	1
Winteranaceæ	1	1
Violaceæ	4	5

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
Flacourtiaceæ	15	31
Stachyuraceæ	1	1
Passifloraceæ	3	4
OPUNTIALES		
Cactaceæ	2	7
MYRTIFLORAE		
Oliniaceæ	*1*	*2*
Thymelæaceæ	*3*	*3*
Elæagnaceæ	3	6
*Lythraceæ**	*3*	*10*
*Sonneratiaceæ**	*1*	*2*
*Blattiaceæ**	*1*	*2*
*Crypteroniaceæ**	*1*	*2*
Punicaceæ	*1*	*1*
Lecythidaceæ	18	45
Rhizophoraceæ	13	32
Nyssaceæ	1	4
Alangiaceæ	1	1
*Combretaceæ**	*7*	*24*
*Myrtaceæ**	*15*	*89*
*Melastomataceæ**	*4*	*9*
*Oenotheraceæ**	*1*	*3*
UMBELLIFLORAE		
Araliaceæ*	25	66
Umbelliferæ	2	2
Cornaceæ*	7	15
ERICALES		
Clethraceæ	1	3
Ericaceæ	8	24
Epacridaceæ	3	9
PRIMULALES		
Myrsinaceæ	8	19
EBENALES		
Sapotaceæ	11	49
Ebenaceæ	2	27
Symplocaceæ	2	6
Styracaceæ	4	8

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
CONTORTAE		
Oleaceæ*	16	59
Nathusia	*1*	*1*
Forestiera	*1*	*5*
Loganiaceæ	*6*	*7*
Apocynaceæ	*15*	*29*
*Asclepiadaceæ**	*1*	*2*
TUBIFLORAE		
Convolvulaceæ	1	2
Polemoniaceæ	1	2
Hydrophyllaceæ	1	1
Boraginaceæ	5	19
Verbenaceæ	12	29
Labiatæ	4	6
Solanaceæ	6	11
Scrophulariaceæ*	6	9
Bignoniaceæ	11	25
Gesneriaceæ	1	1
Myoporaceæ	2	3
RUBIALES		
*Rubiaceæ**	*41*	*78*
Caprifoliaceæ*	5	18
Dipsacaceæ	2	2
CUCURBITALES		
Cucurbitaceæ	1	2
CAMPANULATAE		
Compositæ*	12	21

DIAGNOSTIC VALUE OF VESTURED PITS

In the material examined by me, true vestured pits are either present throughout the secondary xylem of a species or genus or are entirely absent. A similar constancy in the presence or absence of these structures appears to prevail in most families of Dicotyledons. In only four of the 152 families tabulated in Table 1, have I encountered vestured pits in certain representatives of a family and not in others. It should be noted in this connection, however, that in three of the four families the distribution of vestured pits correlates with major subdivisions. Thus, in the Leguminosæ, the vestured pits are present in all the species

and genera examined, with the exception of the Bauhinieæ, whereas in the Euphorbiaceæ they are absent except in the Bridelieæ. They are present in the Exalbuminosæ of the Ochnaceæ, but appear to be absent in the Albuminosæ. In other words, the Oleaceæ, are the only family in which the distribution of vestured pits fails to correlate closely with the systematic classification.

It may be objected that cribriform structures have been reported in representatives of 13 families which are not italicized by me. These families are listed in Table 2.

TABLE 2

Families	Genera and species	Reported by
Fagaceæ	Quercus alba L.	Jönsson (4)
	Q. Cerris L.	"
	Q. pedunculata Ehrh.	"
	Q. obtusiloba Michx.	"
Lauraceæ	Numerous genera and species	Janssonius (3)
Hernandiaceæ	Hernandia peltata Meisn.	"
Hamamelidaceæ	Altingia excelsa Nor.	Moll & Janssonius (5)
Rosaceæ	Cerasus serotinus hort.	Jönsson (4)
	Prunus brigantiaca Vill.	"
Hippocastanaceæ	Aesculus Hippocastanum L.	"
	A. rubicunda hort.	"
Rhamnaceæ	Phylica ericoides L.	"
Guttiferæ	Calophyllum Inophyllum L.'	Ursprung (7)
	C. Calaba Jacq.	Record (6)
Araliaceæ	Hedera helix L.	Jönsson (4)
Cornaceæ	Mastixia trichomata Blume	Moll & Janssonius (5)
Scrophulariaceæ	Veronica Andersoni hort.	Jönsson (4)
Caprifoliaceæ	Viburnum sundaicum Miq.	Janssonius (3)
Compositæ	Helichrysum moniliferum hort.	"

Obviously it is essential to determine whether the "sievelike" appearances reported by Jönsson and others are due to the presence of vestured pits or to artifacts such as are produced during post mortem changes or during the transformation of sapwood into heartwood.

As indicated in Table 1, true vestured pits do not occur in any of the 107 species of Fagaceæ that I have studied. Nor have I succeeded

in finding them in any of the numerous specimens of *Quercus alba, Q. Cerris, Q. obtusiloba,* or *Q. robur* L. (*Q. pedunculata* Ehrh.) that I have investigated. Not only are vestured pits entirely absent in 19 genera and 47 species of the Lauraceæ and in 77 species and 24 genera of the Rosaceæ, but also in numerous specimens of the Prunoideæ, i. e., *Prunus* and its subgenera *Prunophora, Amygdalus, Cerasus,* and *Padus.* Similarly, I have been unable to find vestured pits in *Altingia,* the Bucklandieæ, Altingieæ, Parrotieæ, and Hamamelideæ of the Hamamelidaceæ; in *Aesculus Hippocastanum,* and five other species of the Hippocastanaceæ; in the Zizypheæ and Rhamneæ of the Rhamnaceæ; in *Calophyllum Inophyllum* and other Calophylloideæ of the Guttiferæ; in *Hedera helix* and 65 other representatives of the subgroups, Schefflereæ and Aralieæ, of the Araliaceæ; or in the Mastixioideæ and Cornoideæ of the Cornaceæ.

Although I have failed to find true vestured pits in any of the families listed in Table 2, I have frequently encountered artifacts of various types. In such genera as *Quercus, Altingia, Calophyllum, Hedera, Mastixia,* etc., these artifacts may produce a punctate appearance which closely simulates that of vestured pits. Therefore, in view of the fact that punctate appearances were interpreted as evidence of a sievelike structure, it is not surprising that the species in Table 2 were recorded as having pits with cribriform membranes.

It should not be inferred from this that more detailed and extensive surveys may not reveal the presence of vestured pits in additional families of Dicotyledons. The vestured condition appears to have arisen independently a number of times. To assume that all plants with vestured pits are closely related or are derived from common ancestors which possessed such structures leads to a *reductio ad absurdum.* Vestured pits occur in the more highly specialized types of tracheary tissue and are absent in plants which have a primitive combination of structural characters in the xylem. If vestured pits have arisen independently a number of times, it is not unlikely that genera may ultimately be found in which these structures are present in certain species and are absent in others. It may be inferred, however, from a statistical analysis of Table 1, that sporadic distributions of vestured pits are likely to be of relatively infrequent occurrence in the case of subgenera, genera, and subfamilies.

That vestured pits are extremely useful diagnostic criteria in the systematic study of woods was clearly demonstrated during the course of the present investigation. Most collections of wood specimens, even when accompanied by herbarium specimens, contain a varying number of errors. In other words, the fact that an herbarium specimen and a

sample of wood bear the same number is unfortunately no guarantee that both specimens came from the same tree or that the herbarium specimen was correctly identified. Collections of woods commonly pass through a number of hands and may be subdivided, renumbered, or relabeled. Furthermore, transpositions are likely to occur during the preparation of microscopic slides unless a painstaking system of checking and rechecking is employed. In my reconnaissance of Dicotyledons, I encountered true vestured pits in slides of putative representatives of a number of families which are not italicized in Table 1, and, conversely, ordinary bordered pits in families which are italicized. In all these families, with the exception of the Oleaceæ, the aberrant specimens proved to be errors which could thus be eliminated from the collections.

Similar combinations of anatomical characters occur not infrequently in the woods of families which are widely separated in the systematic classification of Dicotyledons. For example, the secondary xylem of the Osage Orange, *Maclura pomifera* (Raf.) Schneid., so closely resembles that of the Black Locust, *Robinia pseudoacacia* L., that it is extremely difficult to distinguish the woods by anatomical criteria. The woods of the two genera may be identified with certainty, however, by the fact that vestured pits occur in *Robinia* and are entirely absent in *Maclura*.

As indicated in the accompanying text figures and photomicrographs, the number, size, and form of the refractive processes vary considerably in different plants. In certain species and genera the processes are confined to the intervascular pits, whereas in others they may occur as well in the half-bordered pit-pairs, in the fiber tracheids, or upon the inner surfaces of the tracheary walls. Such differences may obviously be utilized as diagnostic criteria in the systematic study and classification of woods.

SUMMARY AND CONCLUSIONS

1. Many of the structures in the tissues of the higher plants which are hypothesized as evidence for the existence of protoplasmic connections can not be interpreted as such.
2. The so-called sievelike appearance of the pits in the vessels of Leguminosæ and of other families of Dicotyledons is not due to perforations of the pit membranes, but to minute outgrowths from the free surfaces of the secondary walls.
3. These refractive processes which vary considerably in size, number, and form are not confined to the pit-chambers in all cases, but may occur on the inner surface of the secondary walls of the vessels.

4. They appear to be restricted to tracheary elements; in half-bordered pit-pairs they are present in the bordered pits of the tracheary elements, but are absent in the simple pits of the adjoining parenchymatous cells.
5. Pits which have these refractive processes may be referred to as *vestured.*
6. In the Dicotyledons (2660 species and 979 genera) examined by me, vestured pits are either present throughout the secondary xylem of a species or genus or are entirely absent. A similar constancy in the presence or absence of these structures appears to prevail in most subfamilies and families.
7. Vestured pits, therefore, are of considerable value both in the systematic study of woods and in discussions concerning the relationships and classification of specific groups of Dicotyledons.

ACKNOWLEDGMENTS

The text-figures used in this paper were drawn by my assistant, Mrs. Ernest C. Abbe. I am much indebted to Professor S. J. Record for his kindness in providing woods of various families of Dicotyledons.

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DESCRIPTION OF PLATES

PLATE 61

Fig. 1. *Prosopis juliflora* DC. Surface view of bordered pits, showing toothed appearance of pit apertures. × 2500.
Fig. 2. *Duabanga moluccana* Blume. Surface view of bordered pits, showing finely punctate appearance. × 1900.
Fig. 3. *Fuchsia Colensoi* Hook. f. Surface view of bordered pits, showing toothed appearance of elongated pit apertures. × 2500.
Fig. 4. *Tibouchina mutabilis* Cogn. Surface view of bordered pits, showing mats of branching and anastomosing processes. × 2500.
Fig. 5. *Combretum species.* Surface view of bordered pits, showing coarsely punctate appearance. × 2500.

Vestured Pits in Dicotyledons

Vestured Pits in Dicotyledons

Vestured Pits in Dicotyledons

Fig. 6. *Eugenia alternifolia* Wight. Surface view of half-bordered pit-pair, showing irregularly punctate appearance. × 2500.
Fig. 7. *Terminalia Chebula* Retz. Surface view of bordered pits, showing coarsely toothed appearance of pit apertures. × 2500.

PLATE 62

Fig. 8. *Combretum species.* Sectional view of pits illustrated in *Fig. 5,* showing coralloid outgrowths from the overarching walls of the pit chambers. × 2500.
Fig. 9. *Parashorea plicata* Brandis. Sectional view of three pairs of bordered pits in the adjacent walls of a vessel (left) and a short tracheid (right). Mats of fine texture fill the entire pit cavities and project more or less into the lumens of the cells. × 2700.
Fig. 10. *Terminalia chebula* Retz. Sectional view of pits illustrated in *Fig. 7,* showing massive projections from the overarching walls of the pit chambers. The imperforate membranes are in the median position. × 3300.
Fig. 11. *Tibouchina mutabilis* Cogn. Sectional view of pits illustrated in *Fig. 4,* showing attachment of mats to overarching walls of the pit chambers. × 2500.
Fig. 12. *Eugenia dichotoma* DC. Sectional view of pit-pair in the adjacent walls of fiber-tracheids, showing toothed inner and outer apertures. × 2500.
Fig. 13. *Parkinsonia Torreyana* S. Wats. Sectional view of half-bordered pit-pairs in the adjacent walls of a vessel (left) and parenchyma (right). The bordered pits are vestured, but the simple pits are not. × 2500.

PLATE 63

Fig. 14. *Bridelia retusa* Spreng. Surface view of vestured inner surface of vessel. × 2500.
Fig. 15. *Sciadodendron excelsum* Griseb. Surface view of bordered pits, showing artifacts which simulate the deeply-staining processes of vestured pits. × 2500.
Fig. 16. *Acacia flexicaulis* Benth. Sectional view of wall of vessel, showing vestured inner surface. × 2500.
Fig. 17. *Duabanga moluccana* Bl. Sectional view of pits illustrated in *Fig. 2,* showing mats of fine texture within the pit chambers. × 1900.
Fig. 18. *Eugenia alternifolia* Wight. Sectional view of half-bordered pit-pairs in the adjacent walls of a vessel (right) and parenchyma (left). The bordered pits are vestured, but the simple pits are not. × 2500.

ARNOLD ARBORETUM,
HARVARD UNIVERSITY.

SPECIES HYBRIDS IN PLATANUS AND CAMPSIS

KARL SAX

With two text figures

ACCORDING TO SEWARD (1931), *Platanus* is one of the oldest of broad-leaved trees, and fossil types have been found which are very similar to the fertile branches of modern species. This genus was one of the most widely spread of the earlier cretaceous dicotyledons. The cretaceous *Platanus* was more variable and had a much wider distribution than the existing species.

The genus *Platanus* is now found in North America to Mexico and from southeastern Europe to India (Rehder, 1927). The American species, *P. occidentalis,* is hardier than the Old World species, *P. orientalis;* but the two species must be rather closely related because they have produced the fertile hybrid *P. acerifolia.* According to Henry and Flood (1919), *P. acerifolia* originated as a natural hybrid between the Occidental and Oriental Plane. It probably originated in the Oxford Botanic Garden about 1670. The hybrid is intermediate in leaf and fruit characters and seems to possess unusual vigor. The hybrid is extensively used for planting in the streets of European towns where neither of the parental species will survive. Seedlings from the hybrid are variable, some of them resembling the parental species.

The fact that the species hybrid is fertile, and segregates in the second generation, would indicate that the parental species have the same number of chromosomes and that their chromosomes are similar. The haploid chromosome number of *Platanus* has been reported to be 21 (Winge, 1917), 8 (Brouwer, 1924), and 20-22 (Bretzler, 1924). Permanent smear preparations of pollen mother cells from *P. occidentalis* and *P. acerifolia* show clearly that the number of chromosomes is 21, as reported by Winge. The chromosomes are paired regularly at the first meiotic division, and there is no evidence of lagging chromosomes at any stage in the meiotic divisions. The chromosomes at the first meiotic metaphase, and at telophase, in the hybrid, are represented in Figure 1. One of the chromosomes is somewhat smaller than the others, especially when fixed in aceto-carmine solution. The chromosomes are too small and numerous to permit an accurate determination of chiasma frequency, but both rod and ring chromosomes were observed. The average chiasma frequency is about 1.5 per bivalent. About 90 percent of the pollen is good in the hybrid.

The apparent compatibility of the chromosomes from the two parental species in the first generation hybrid indicates that the Old and New World species have undergone no very fundamental changes since their segregation and differentiation. Although this genus has undergone no very fundamental changes for a long period of time, and even though it is one of the oldest dicotyledons so far discovered, it does not possess characters which mark it as a primitive type or as an early member of an evolutionary series (Seward, 1931). It is of interest that *Platanus* is the only genus of the family Platanceæ.

Another valuable hybrid between species from the Old and New World is *Compsis Tagliabuana* (*C. hybrida*) (Rehder, 1932). The parental species are *C. radicans*, from central and southern United States, and *C. chinensis*, from China. The American species seems to

FIGURE 1. Meiotic chromosomes of *Platanus acerifolia* at metaphase and telophase. The 21 bivalents divide regularly. × 2000.

be hardier and more vigorous than the Chinese species, but the flowers of *C. chinensis* are more attractive. The hybrid has the good qualities of both parents. It is almost as attractive as the Chinese species and has the hardiness of the American species. The hybrid forms are grown in many gardens of southern Massachusetts (Anderson, 1933).

The hybrid was first recorded by Visiani as having been raised by the brothers Tagliabue apparently some time before 1859, but doubtless it has originated independently elsewhere afterwards or even before 1859. Natural hybrids have also been obtained at the Botanical Garden in Washington, D. C. A more complete account of the origin and characteristics of *Campsis Tagliabuana* has been presented in the Arnold Arboretum bulletin of popular information, by Edgar Anderson.

The hybrids are partially fertile, and numerous segregates have been produced. The chromosome number is undoubtedly the same for both the parental species and the hybrid. There are 20 pairs of small chromosomes at the first meiotic division in *C. radicans*. No representa-

tives of *C. chinensis* were available for study, but a hybrid segregate much like the Chinese species, also has 20 pairs of chromosomes. The hybrid has 20 pairs of chromosomes which are perfectly regular in pairing and disjunction at meiosis. The chromosome number found in the hybrid is in accord with the count reported by de Vilmorin and Simonet (1927). The chromosomes are very small and usually form rod bivalents at the first meiotic division. The chiasma frequency is apparently little more than one per bivalent.

FIGURE 2. Meiotic chromosomes of *Campsis radicans, C. Tagliabuana* and *C.* spec. (probably a hybrid segregate resembling *C. chinensis*). The divisions are regular in the one parental species examined and in the hybrids. × 2000.

Although there is regular pairing and disjunction of the chromosomes in *Campsis Tagliabuana,* about 50 per cent of the pollen is sterile. There is less than 5 per cent pollen sterility in *C. radicans.* Pollen sterility in species hybrids which have regular meiotic divisions is also found in other genera (*Primula kewensis,* diploid, Newton and Pellew, 1929; *Deutzia* and *Philadelphus,* Sax, 1931; *Tradescantia,* Sax and Anderson, 1933). Pollen sterility in such species hybrids might be caused by unequal interchange of chromosome segments in one of the parental species. The interchange of segments between non-homologous chromosomes has been found in a considerable number of different genera in slightly related families, and may be much more extensive than is indicated by the formation of rings or chains of chromosomes. In a species with a low chiasma frequency, segmental interchange would not result in chromosome rings, and if segmental interchange were unequal, chromosome pairing would be regular in both heterozygous and homozygous forms. The heterozygous types will be eliminated unless they possess a much greater survival value to compensate for their partial sterility or unless balanced lethals are involved. As a result, plants should originate which are homozygous for segmental interchange chromosomes, as is known to be the case in *Datura, Pisum,* and *Oenothera.*

If an individual homozygous for two segmental interchange chromosomes (the minimum number possible) is crossed with a normal plant, the chromosomes should pair as bivalents and divide normally if the

segmental interchange is unequal and if the chiasma frequency is low. But the random segregation of the chromosomes would result in 50 per cent non-disjunction of a chromosome segment. If both segments are essential for gametophyte development, the pollen sterility would be 50 per cent; if only the longer segment is essential, the pollen sterility would be 25 per cent. Pollen sterility would be almost complete in a plant heterozygous for four or five segmental interchange chromosomes.

Segmental interchange might well be one of the factors involved in the differentiation of species. A form with relatively few segmental interchange chromosomes would tend to be isolated from the normal type because of the sterility of the F_1 hybrid between the two forms. Unless lethal factors are associated with the segmental interchange chromosomes, the homozygous forms should have a higher survival value. Variations originating in the different lines homozygous for chromosome structure would not be swamped by intercrossing and would tend to be developed more or less independently in different structural genoms.

SUMMARY

Platanus acerifolia, a natural hybrid between *P. orientalis* of southeastern Europe and *P. occidentalis* of North America, has 21 pairs of chromosomes which pair regularly at meiosis. The hybrid is fertile, and genetic segregation occurs in the second generation. These facts indicate that the parental genoms are similar and are compatible with each other, even though the parental species have been isolated for a long period of time.

Campsis Tagliabuana, a natural hybrid between *C. chinensis* from China and *C. radicans* from North America, has 20 pairs of chromosomes which pair regularly at meiosis. Although the reduction divisions are regular, there is about 50 per cent pollen sterility in the F_1 hybrid. The association of regular chromosome pairing and partial or nearly complete pollen sterility in species hybrids may be the result of segmental interchange between non-homologous chromosomes in one or both parental species.

The species hybrids in the above genera contain the desirable characters of the parental species and are especially valuable because of their hardiness. Such hybrids are good illustrations of what may be expected from many hybrids between Old and New World species.

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CYTOLOGICAL LABORATORY, ARNOLD ARBORETUM,
HARVARD UNIVERSITY.

CHROMOSOME BEHAVIOR IN CALYCANTHUS

KARL SAX

With four text figures

TWO SPECIES of *Calycanthus* are found in eastern United States. *Calycanthus floridus* L. is found from Virginia to Florida while *C. fertilis* extends from Pennsylvania to Georgia and Alabama (Rehder, 1927). Herbarium material in the Arnold Arboretum includes *C. fertilis* from eleven localities in North and South Carolina and Georgia, and *C. floridus* from fifteen localities in South Carolina, Georgia and Alabama. The ranges of the two species overlap to some extent, but there is little evidence of hybridization although the two species are similar, differing chiefly in leaf characters. Varieties of the two species have been described, but in the case of *C. floridus* at least, the variety (*ovatus*) is rare and is apparently known only in cultivation, and appears to be of European garden origin.

Chromosome counts were obtained from one plant of *C. fertilis*, two plants of *C. floridus*, and two varieties of these species. In both species there are eleven pairs of chromosomes at meiosis. The homologous chromosomes are united by terminal or subterminal chiasmata. The chiasma frequency is somewhat less than two per bivalent at early metaphase. Some of the chromosomes are apparently heterobrachial and the separation of the short arms at late metaphase and early anaphase gives the impression of a prevalence of rod bivalents at the later stages although at early metaphase most of the chromosomes are ring bivalents. The chromosomes of *C. fertilis* are shown at early metaphase (Fig. 1) and those of *C. floridus* are shown at late metaphase of the first meiotic division (Fig. 2).

A variety of *C. fertilis* also had eleven pairs of chromosomes which pair and divide regularly at meiosis. The other variety in the Arboretum, *C. floridus ovatus*, is a triploid. At meiosis there are often eleven trivalents, although from one to four univalents are usually found. The trivalents are found in the form of chains, rings and rods, and Y's (Fig. 3). There is some irregularity in the first meiotic division, including both trivalents and univalents, and the chromosomes are distributed irregularly to the poles. The chromosomes at second metaphase following a comparatively regular division are shown in Figure 4. In one case a distribution of 20-13 was observed, but as a rule the numbers of chromosomes passing to each pole are approxi-

mately equal. Occasionally more than thirty-three chromosomes are found at the two poles, due presumably to a precocious division of one or more univalents.

The pollen sterility of the triploid is about fifty per cent as compared with about five per cent in each of the pure species.

Figure 1. CALYCANTHUS FERTILIS: chromosomes at early metaphase of the first meiotic division.—Figure 2. CALYCANTHUS FLORIDUS: late metaphase.—Figure 3. CALYCANTHUS FLORIDUS OVATUS: a triploid showing trivalent chromosomes at meiosis.—Figure 4. The triploid variety showing chromosome distribution at the second meiotic division.—The figures were drawn from aceto-carmine preparations. Magnification × 1200.

Since the two species of *Calycanthus* are similar in taxonomic characters and overlap in their distributions, the occurrence of natural hybrids might be expected. Some of the diploid varieties may be of hybrid origin, but there seems to be no extensive hybridization, and the two species are rather well differentiated. It is possible that tetra-

ploid forms of these species exist and that the triploid variety is a hybrid between a tetraploid, *C. floridus* and a diploid *C. fertilis*, but it seems more probable that the *ovatus* variety is an autotriploid. The two plants of *C. floridus* in the Arboretum are typical for the species and both are diploids. The variety *ovatus* originated, or was first found, in a European garden and is not known to occur in nature. No species of *Calycanthus* is a native of Europe.

Chromosome irregularity and pollen sterility have been considered as evidence of hybridity. In the case of *Calycanthus floridus ovatus* it is improbable that chromosome irregularity and pollen sterility can be attributed to species hybridization. An autotriploid originating within a species would be expected to show the chromosome irregularities and pollen sterility. Chromosome irregularity at meiosis and pollen sterility can also be caused by segmental interchange with absolutely no change in the taxonomic characters of the plants involved. *Tradescantia edwardsiana*, for example, is a well marked species. The occasional segmental interchange plants show about fifty per cent pollen sterility, although they are taxonomically the same as the normal fertile plants (Sax & Anderson 1933).

Chromosome irregularities may also be caused by variations in temperature and by genetic factors. On the other hand species hybrids often show regular chromosome pairing and division. In some of these hybrids there is much pollen sterility, but others are relatively fertile. Undoubtedly wide species crosses often result in hybrids which exhibit irregular chromosome behavior at meiosis, but chromosome irregularity is not necessarily evidence of species hybridization.

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Arnold Arboretum,
Harvard University.

ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

HAIG DERMEN

With plates 64 to 67

TABLE OF CONTENTS

A. INTRODUCTION

THE PROBLEM of the nucleolus is one which has attracted investigators for many years, but despite the large amount of work done on this nuclear body, the problem is far from solved. The origin, the development, the relation between nucleolus and chromosomes, and the nucleolar function are phases of the problem which have received attention, but there is little concerted opinion regarding them. That the nucleolus is an essential component of cells is evidenced by the fact that it is present, very probably, in all types of cells of all organisms, a fact which may be responsible for Wilson's question (1925) "whether the nucleolus may not play a more active and important part in cell metabolism than most writers have hitherto assumed."

The importance of a cell body is not necessarily dependent upon its continuity and permanence in a particular structural form, such as is characteristic of the chromosomes. Unlike the chromosomes the nucleolus possesses no constant structural form and is not even continuous, in the strict sense of the word, appearing and disappearing during both mitosis and meiosis. This peculiarity alone has perhaps caused a confusion in evaluating its significance, but when its universality is considered, it would appear that its function is one of vital import to the cell, and its appearance and disappearance in the cell cycle may be correlated with that function, which is probably of a metabolic nature.

The present work is primarily intended to determine the origin and to trace the development of the nucleolus through all the stages of mitotic and meiotic development of the nucleus in plants of diverse types and to see what relationship exists between the chromosomes and nucleolus.

Many of the recent investigations are concerned with the origin of the nucleolus and its relation to the chromosomes. Its function has been, and still is, a matter of speculation. Van Camp (1924) believes that nucleoli originate from the chromosomes at telophase in the form of small globules which later fuse into larger forms, but still later, during the development of the chromosomes, through a direct contact established between a continuous spireme and the nucleolus, the nucleolar substance flows back again over the chromosomes to be again dissociated during telophase. Zirkle (1928 and 1930), by developing specific fixatives, confirms the idea of direct transportation of nucleolar substance into the spireme and demonstrates the presence of this substance in a skeleton form in the anaphase chromosomes. Heitz (1931a and 1931b) offers evidence to show that the nucleolus originates from the satellite chromosomes and only from them, and formulates a theory

that accounts for the symmetry of the nucleoli in number, size, position, and form in the telophase of the sister* (daughter) nuclei. Abele (1930) seems to believe that the nucleolar substance divides equally, as do the chromosomes, thus giving rise to nucleoli in sister nuclei that show symmetry in all respects and that "jeder Nukleolus hat seinen Partner in der anderen Tochterzelle."

As for the function of the nucleolus, Montgomery (1898) assumes that the nucleolar substance is connected with the nutritive processes of the cell and, as an assimilation organ, takes food from the cytoplasm. Meyer (Guillermond et al., 1933) believes that the nucleolus is composed of "ergastique" elements of the cell. It is commonly believed that the nucleolus is nothing but reserve food for the chromosomes which may build themselves from this supply. De Mol (1926), Zirkle (1928), and Fikry (1930) suggest that this body may break up into small particles and act as the carriers of hereditary stimuli from the genes through the cytoplasm. Further discussion of these theories, as well as others, will be found in the text.

In addition to a study of the origin and behavior of the nucleolus an attempt has been made in the present study to clarify some of the points concerning the structural behavior of the chromosomes as a group throughout their developmental cycle and to throw light on the question of the supposedly continuous spireme and on the physical relationship often observed between chromosomes and nucleolus. Some of the conclusions are based on observations made from both living and fixed material.

B. MATERIAL AND METHODS

To make a study of this nature as comprehensive as possible, it was thought essential that the observations be made on various plants that are unlike taxonomically. The following plants were used: (a) *Callisia* and *Yucca* (Monocotyledons); (b) *Paeonia* and *Hepatica* (Dicotyledons); (c) *Pinus* (Gymnosperm); (d) *Polystichum* (Pteridophyte); and (e) a species of *Spirogyra* (Thallophyte). Some observations were made from various other plants to see if there could be found any peculiarities not present in those mentioned above. The most intensive observations were made on *Callisia* and *Paeonia*.

It was thought advisable to supplement the study of fixed material with that of living material as an aid in interpreting the phenomena observed. Living material was subjected to various toxic fluids, and

*The use of the terms "sister cells" and "sister nuclei" is suggested when cells or nuclei of the same "generation" are discussed, "daughter cells" and "daughter nuclei" being reserved for cases where derivation is implied. These terms are so used in this paper.

their effects on the protoplasm in general, and nucleolus in particular, were observed, and thus an interpretation of fixed objects could be arrived at.

A fixative of 5% formalin and .5% chromic acid, in equal parts, was the principal killing and fixing solution used. This fixative seems to have been originally developed by Lewitzky (1931) and was recommended by Marshak (1931). The present author has made use of it with success. Its usefulness was proved by applying it to the delicate stigma hairs of *Callisia,* and it was found to cause less disturbance to the cell and its contents than any other reagents commonly used for cytological preparations. With this fixative the nucleolus takes a dark stain both with crystal violet-iodine and iron-alum haematoxylin. In the material fixed with this reagent and stained with crystal violet-iodine both chromosomes and nucleolus retain the stain remarkably well even after long washing in absolute alcohol. For the technical procedure with this fixative the reader is referred to my paper (Dermen, 1932). In addition to this method of preparation the Ehrlich-Biondi differential stain recommended by Van Camp (1924) was used. This is made up of Orange G, acid fuchsin, and methyl green. (For full details the reader is referred to Lee's Vade Mecum, p. 177, 1928). Blotting paper tests of this stain described in this book were found essential and, accordingly, the proportion of reagents could be varied to give the proper reaction.

C. MORPHOLOGY OF CHROMOSOMES IN RELATION TO THE NUCLEOLUS

In order to make clear the nature of the relationship which may exist between chromosomes and nucleolus, it was considered essential that first a thorough analysis of the chromosome morphology should be made. For this reason this phase of the problem was undertaken.

I. Structural Continuity of Chromosomes in Cycle. When the Lewitzky fixative is used, at no period of development are the chromosomes found aggregating into an unrecognizable mass. They always retain their linearity, and at prophase are found within the nuclear membrane as units grouped in the same relative positions which they occupied during anaphase. It seems that the individual chromosomes retain their particular forms (Figs. 48, 49, and 2) as they move to their respective poles during division, bent at their polar constriction points. The prophase chromosomes (Fig. 2) differ from those at anaphase (Fig. 49) only in that the former are more opened up to fill the space inside the nuclear membrane, while the latter are forced together somewhat into a more compact form in moving to the poles. This

compactness is extreme at telophase (Figs. 6, 10, 11, 16, and 17). From this point on, as soon as the new nuclear membrane is formed, there take place reverse morphological changes. A new nuclear membrane encloses the closely grouped telophase chromosomes; then follows the expansion of this membrane, due possibly to expansion of the tightly pressed chromosomes which start to develop into longer and finer threads, to the increase of nuclear sap, and to the development of nucleoli in number and size, resulting in an increase of nuclear volume as a whole. In comparing specifically Figs. 2 and 49 (in Fig. 49 only upper polar group should be considered), prophase and anaphase respectively, one can not fail to be impressed by the similarity of the orientation of the chromosomes. The difference is that in Fig. 2 the chromosomes are longer, spiral and zigzag in appearance, while in Fig. 49 they are short and straight, tending to crowd together as they are forced to the pole, and appear single in structure. It was found, however, after critical study, that even at anaphase the chromosomes possess a dual structure. As in prophase (Fig. 2) and early metaphase (Fig. 29—in this figure the region of primary constriction is illustrated as faithfully as possible), anaphase chromosomes are found split lengthwise and even show twists in the chromatid threads, as illustrated in Figs. 7 and 30, and these twists become more pronounced in telophase, as shown in Fig. 8.

The chromosome contours at anaphase, as illustrated in Figs. 49-51, seem significant, and the interpretation may be that these chromosomes are split and their chromatids twisted together, as shown in Figs. 7, 8, and 30. Similarly, premeiotic chromosomes of microsporocytes show similar contours, indicating that they are already split before microsporocyte chromosomes go through characteristic meiotic development. The split nature of the chromosomes at this stage has been confirmed by a number of reputable cytologists, such as Sharp (1929) and Kaufman (1931), working with plants, and McClung (1928) and Robertson (1931), working with animals. Darlington (1932), however, still maintains that duality of the chromosomes at this stage is due to optical illusion, although previously (1926) he reported seeing chromosomes split at anaphase. This anaphasic split, as maintained by Sharp and others, seems to take place during the late prophase and metaphase. Such a split was seen in the Callisia satellite chromosomes, as illustrated in Fig. 5. The chromosomes at this stage, besides being fully split into distinct halves in readiness for mitotic division, also seem to show in each chromatid a secondary split, part of which, at least, could be distinguished if observed carefully. At the upper end split there were also observed chromomere-like structures in pairs. It may be remarked

here that only rarely can these features be made out clearly. Delicate fixation and staining are primary requisites; and even with the best technique it requires special attention and considerable care on the part of the investigator that this feature may not be interpreted as an optical illusion.

Here may be considered the arrangement of the chromosomes on the metaphase plate in preparation for mitotic division. The metaphase chromosomes (*Callisia*, Fig. 48) have a characteristic orientation which is considered normal; they are arranged in circular fashion, polar (primary) constrictions pointed toward the middle of the plate, and homologous chromosomes tending frequently to show a secondary pairing. If homologues are not always adjacent, at least there seems to be a tendency for them to orient themselves in such a fashion as to facilitate synapsis during meiosis. There may be a strong affinity between homologues at their polar constriction points that will play some part in bringing them together and result in their close pairing at meiosis. Structurally the chromosomes of *Callisia* vary considerably. The total number, as reported by Sax (1932), is 12, of which 4 have approximately median constriction (Figs. 48 and 49), and 8 have subterminal constriction. Two of the latter possess satellites. No secondary constrictions are observed.

It is not uncommon to find in the literature references to a granular stage of nucleus during the resting stage. A careful study of Figs. 18 and 33 perhaps will throw some light on the exact nature of the nucleus at this stage. Fig. 33 represents the telophase of somatic chromosomes from *Paeonia*. The two sister nuclei are viewed from the side and illustrated more or less diagrammatically. At this stage, as was pointed out above, the chromosomes are found already split and showing fine threads, each one representing a chromatid. The appearance of granulation often observed at these stages seems to be due both to curvatures and to points of strongly stained areas (Fig. 18). In mitosis these chromosomes will further elongate and open up to develop finally into a structural make-up similar to that illustrated in Fig. 2. If this stage were viewed from a different angle, it would no doubt present an entirely different aspect. Therefore, if it is true that at prophase the chromosomes hold positions comparable to those at anaphase, then any possibility of their joining end to end into a continuous spireme is inconceivable. This point is further confirmed by the work of Sax and Anderson (1933) and others who show that chromosomes may interlock during meiosis, a situation which would be impossible if the chromosomes were continuous.

The conception of the continuity of the chromosomes in a spireme

has probably been derived from impressions of oblique and polar views of nuclei of stages illustrated in Fig. 1, and especially of early stages similar to those in Fig. 18. (Fig. 1 is of the same stage as Fig. 2, except that in the former the nucleolus is included.) Even in a stage as late as Fig. 1 one can easily be misled concerning the continuity of the chromosomes, but with care they can clearly be made out as separate units. When root-tip sections were crushed under a cover-glass to flatten the cells, it was apparent that in all stages chromosomes invariably show linearity and are never broken up into scattered granules. Martens (1928), having made a similar study from living and fixed material, finds the nucleus is not granular in make-up but primarily reticulate and filamentous, and that there is no continuous spireme at the beginning of prophase; that "Le réseau interphasique n'est donc que l'ensemble des chromosomes télophasiques—à peine plus évolués—et donc les mailles filamenteuses sont reliées par d'autres filaments d'union," and confirms "la persistance—ou mieux, la continuité morphologique et génétique—des structures chromosomiques d'une cinèse à l'autre, au cours de l'interphase et du repos."

II. Chromosome and Nucleolar Relationship. It can be seen from the preceding description of chromosomes that the idea of a continuous spireme is incompatible with the facts, that the chromosomes retain a characteristic arrangement inside the nuclear membrane, and that the individuality of the chromosomes is maintained; therefore a direct flow of nucleolar material, postulated by Van Camp and many others, from the end of a chromosome through the rest of the chromosomes, becomes an impossibility.

Figure 1 shows a large central nucleolus around which the chromosomes are distributed. In this nucleus all the chromosomes are found, with one exception, free from any physical connection with the nucleolus. At the upper side of the nucleolus there was noted the only probable connection at the bend of a chromosome with the nucleolus. I was not able to discover, at this stage, the presence of a pair of satellites characteristic of this species of *Callisia* and determine their relationship with the nucleolus, and only once, after a long search, I found two satellite-like bodies, one on each side of the nucleolus, at a considerable distance from the chromosome ends viewed from the side of the nucleus in a position similar to the one illustrated in Fig. 2; however, these bodies may have been extra-nucleolar bodies which are described below.

Van Camp (1924), using the Ehrlich-Biondi differential stain, found an intermediate coloration on the portion of the chromosomes attached to the nucleolus and therefore concluded that there was a direct flow

of nucleolar material over the chromosomes. Zirkle (1931), by using a selective fixation method, confirms this conclusion and finds that the nucleolar material which has flowed "into" the chromosome threads was fixed at anaphase in a skeleton form. The present author has been unable to confirm the findings of these investigators. In all the cells he has examined there was no sign of intermediate staining between any portion of the chromosomes and nucleolus. The nucleolus at all stages was always bright red and the chromosomes pale blue when material was stained with the Ehrlich-Biondi solution. Fig. 3 is a diagrammatic drawing of a prophase stage from differentially stained material where no such intermediate coloration could be observed. On the other hand, if Zirkle's contention is correct, then the anaphase chromosomes were expected to show some red granular framework. Fig. 6 is such an anaphase stage; the chromosomes were of the same pale blue color as in the prophase in Fig. 3. When *Paeonia, Pinus, Polystichum,* and other species were subjected to similar treatment, the results were the same as those found in *Callisia;* the differentiation was always perfect.

It is quite generally assumed that the nucleolus decreases in volume during the development of the chromosomes, and that it supplies material to the "emerging" chromosomes. The facts on hand, however, seem to indicate that, at least in the plants studied, the situation is not such as described above, and that there is no diminution in size of the nucleolus from the resting stage, when it reaches its maximum volume, until the end of prophase before the nuclear membrane disappears. This fact can be demonstrated clearly by studying meiotic stages of species in which only one nucleolus is generally found, derived, no doubt, from fusion of a number of small nucleoli to form a large one. In *Callisia* there are usually two nucleoli present in the earliest meiotic stage, but as a rule there is only one at later stages. When there are two at early leptotene stage (Fig. 19), they are always smaller than the single nucleoli in adjacent nuclei (Fig. 20). Even at these early stages there are very few cells with double nucleoli, and these unquestionably later result in one nucleolus by fusion. Figs. 21-24 represent the zygotene stage, where the chromosomes are considerably thicker than in Fig. 20 and Fig. 25 the satellite chromosome pair at late diakinesis just before the nuclear membrane disappears, which, in this species, always holds the nucleolus at its satellite end, as reported by Sax (1932). In comparing the sizes of nucleoli in Figs. 20 and 25 and comparing both with the inner nucleolus of Fig. 18, which represents a cell from the earliest stage of meiotic development resulting from the division of the archespores, one is impressed by the constancy in size of the nucleoli all through these developmental stages. Similarly, Fig. 57

shows the diagrammatic representation of the volumes of the two nucleoli from *Hepatica, a* sketched from a very early leptotene, and *b* from the diplotene stage, showing the same constancy in size of the nucleolus as in *Callisia.*

The assumption of transference of nucleolar material to the chromosomes seems to be based on the fact that at later stages the chromosomes take up stain more readily than at earlier stages, a fact which has led many investigators to believe that there is a correlation between the stainability of chromosomes at later stages and entire disappearance of the nucleolus by the time of chromosome division. In *Yucca,* illustrated in Figs. 55 and 56, it is shown that there is hardly any sign of decrease in volume of the nucleolus which is left out in the cytoplasm during meiosis. In general, it appears that the nucleolus reaches a maximum size at a very early stage in the nucleus soon after a nuclear membrane encloses the chromosomes; the size of the nucleolus remains constant all through further development of the nucleus to the very end of prophase, very soon after the nuclear membrane disappears; then the nucleolus generally disappears, the rate depending upon the species of plant, as will be indicated below.

Belar (1928) seems to be justified in stating that there is not sufficient ground for assuming that the nucleolus has any role in the process of building up the chromosomes by increasing their mass. It was shown that when the differential stain was applied at all stages, the chromosomes took a pale blue color, while the nucleolus stained bright red. On the other hand, when crystal violet-iodine, or even iron-alum haematoxylin, is used, the chromosomes throughout do not show the same intensity of coloration as when Ehrlich-Biondi stain is used. Therefore this discrepancy of staining intensity of the chromosomes does not seem to be due to their being less chromatic at earlier stages, and more so at later stages, but rather to their "thinness" or "thickness," depending upon their stage of development. Moreover, the stainability and the degree of retaining of the stain of the fine chromosome threads may be altered with different fixatives and stains.

III. Satellites and Secondary Constrictions. Belar (1928) states that there is no basis for assuming that there is any connection (physiological) between satellites and nucleoli, while, on the other hand, Heitz (1931a and 1931b) has given great importance to these bodies. Heitz's theory is that nucleolar bodies originate on and around the achromatic threads behind the satellites and at secondary constriction areas, and that the number of nucleoli at late telophase is the same as these satellite and constricted chromosomes. De Mol (1927) was perhaps the first to notice a correlation in the number of satellites and

nucleoli. In the present paper is reported a complete study of the satellite and constriction situation in *Callisia, Paeonia,* and *Pinus,* in order to have a basis for analyzing the relationship of the nucleolus to these features.

The somatic chromosomes of *Callisia, Paeonia,* and *Pinus* were subjected to a detailed study to determine the presence and number of satellites and secondary constrictions. Pinus chromosomes were found to be devoid of typical globular satellites, but instead there were present five pairs of chromosomes with secondary constrictions which varied as to their position and length of achromatic threads, though apparently constant for each chromosome so characterized. Fig. 54 illustrates *Pinus Strobus* chromosomes at anaphase (only part of the 12 pairs of chromosomes in each half is shown), showing some of these constricted areas on the chromosomes. *Callisia* (Fig. 48) has a pair of small satellites attached to relatively long threads which were hardly noticeable in this figure but are generally of good size, as shown in Fig. 5. Four plants of *Paeonia* were studied, two of which were of the species *P. suffruticosa* and two of different species,—namely, *P. Delavayi alba* and *P. Woodwardii.* Fig. 50 (*a* and *b*), drawn from two adjacent sections, represents an anaphase from *P. suffruticosa* which was cut into two by the microtome knife. The exact number of the satellites in this species was determined from this, as well as from other cells, and was found to be four, the position of which can be clearly seen. Two satellites are present on the short arms of a pair subterminally constricted, and the other two on the short arms of a submedianly constricted pair. In Fig. 50b in the lower polar group one satellite was distinctly split, indicating a behavior similar to chromosomes at this stage discussed earlier. Here it should be emphasized that the satellites are normal components of some chromosomes and form their characteristic features with permanent attachment and are not free bodies picked up by chromosomes from the surface of the nucleolus, as was suggested by Navashin (1927).

The *Paeonia* species studied had 5 pairs of chromosomes. They varied somewhat in size of chromosomes and satellites and in number of satellites. Fig. 50 represents a young seedling of *P. suffruticosa* from the Arnold Arboretum, while Fig. 51 represents a plant of the same species that has been growing at the side of the Bussey greenhouse. When slides made from these two plants were compared, a constant difference in number of nucleoli was found. The highest number reached in the greenhouse plant (Fig. 51) was always less than that of the Arboretum plant (Fig. 50). On the basis of Heitz's theory it was suspected that this discrepancy in the number of nucleoli in two differ-

ent plants of the same species might be due to a difference in the number of satellites. To test this idea many cells at anaphase and metaphase were crushed by pressing on the cover glass and forcing the chromosomes to spread apart to make their satellited feature more obvious. Strikingly enough, the result was that actually the number of satellites of the plant near the greenhouse was less than in the plant of the Arboretum, three and four respectively. Fig. 51 represents an anaphase stage with three satellite chromosomes at each pole pressed flat and the chromosomes forced to spread. There was one pair of chromosomes submedianly constricted with very small satellites compared with a similar pair referred to in Fig. 50, and only one of the chromosomes with a subterminal constriction possessed a satellite corresponding in size to the ones illustrated in Fig. 50.

Figure 52 is a metaphase stage of *Paeonia Woodwardii* chromosomes. This plate also was crushed and flattened. The division at the polar constriction areas was quite distinct. There were six satellites, two more than in *P. suffruticosa* (Fig. 50), these two additional ones being on the shorter arms of a near-medianly constricted pair of chromosomes, while the other two pairs were on the same type of chromosomes as in *P. suffruticosa*. The satellites on these sub- and near-medianly constricted chromosomes were as large as the satellites of the submedianly constricted chromosomes of Fig. 50, while the ones on the subterminally constricted chromosomes were very minute and close to the end of the chromosomes and were determined only after considerable effort. Fig. 53 is from *P. Delavayi alba*, with one subterminally constricted chromosome being shown which possessed a large satellite quite comparable to the ones in Figs. 50 and 51, in contrast to the small ones in Fig. 52, varying only in the length of the achromatic thread, which was somewhat shorter. In other details the chromosomes of this species were comparable to those of *P. Woodwardii* (Fig. 52). On the basis of polar constriction the chromosomes of all *Paeonia* species reported here may be classified thus: one pair subterminal, one pair submedian, and three pairs (in varying degree) near-median.

D. ORIGIN AND BEHAVIOR OF THE NUCLEOLUS

Considerable space has been given to consideration of two important works by Van Camp (1924) and Heitz (1931a and 1931b) concerning the origin of the nucleolus in plants. Van Camp, applying Ehrlich-Biondi stain, was able to show that the nucleoli originate from the chromosomes at telophase in the form of small globules which later, by fusion, collect into one large nucleolus, while Heitz limits the origin of the nucleoli to the satellite chromosomes, specifically on and around

the achromatic threads that connect either a satellite or a constricted arm with its chromosome. These two findings were subjected to an intensive study and their merits evaluated.

I. The Nucleolus in Somatic Cells. The origin of the nucleolus is best studied in longitudinal root-tip sections of plants with large chromosomes. Fig. 9 represents two sister cells from Callisia root-tip sections. Here are shown, diagrammatically, small globules of nucleolus that were differentially stained red in contrast to the pale blue color of the chromosomes which are not shown. These globules collect, undoubtedly by fusion, into two and finally one body (Figs. 10 and 11) and, as more globules are produced and added together, the nucleolus grows very rapidly and becomes constant in size during further development of the chromosomes. In general, the number of nucleoli in *Callisia* is one or two, and quite rarely three, as illustrated in Fig. 12. All these three nucleoli were homogeneously colored bright red by the Ehrlich-Biondi stain, indicating that they were of the same nature. Whenever it was possible to distinguish the outlines of the chromosomes differentially stained, it was observed that these globules were on the surface region, around and between the split chromosomes. Fig. 31 is from *Paeonia suffruticosa* (greenhouse plant). The granules were found not only where the chromosomes are more compact, but also on the arms of the chromosomes that lay outside the region of the compact mass. Here it can be clearly shown that the origin of nucleolar substance is not associated with the achromatic region of the satellite. Otherwise, assuming that these exposed arms may represent satellite arms, there should have been one granule to an arm, which is not the case. Fig. 32 is a diagrammatic representation of a similar stage, drawn on a larger scale to bring out the approximate number of these globules. Fig. 37 is from *Pinus* and represents the same very high number of small nucleolar globules. In *Polystichum* was found the same situation as in others; however, the higher number of chromosomes in this species ($2n = 60+$) making it difficult to study the satellite or secondary constriction (if present), the representation of this situation was limited only to plants in which the number of satellites and secondary constrictions was determined so as to have a sound basis for discussing satellite and nucleolar relationship. Because of the same difficulty, a species of *Arisaema* (*A. triphyllum*, collected near Pepperell, Mass.) with $2n = 60+$ chromosomes is not illustrated, but this also showed the same numerous small globular nucleoli at a stage similar to that illustrated for *Paeonia* and *Pinus* in Figs. 32 and 37 respectively.

In Fig. 29 is shown a part of a satellite chromosome from an early metaphase plate of *Paeonia suffruticosa* (Arboretum plant) to illustrate the supposedly true relationship between a satellite and the nucleolus. This small nucleolus is, perhaps, a remnant of a larger nucleolus that has not completely disappeared in the cytoplasm. It can be seen not only that this nucleolar body does not surround the achromatic thread—contrary to Heitz's theory—but also that it lies away from the thread and is found very near the satellite (there may actually be an attachment between them). Both McClintock (1931) and Burnham (1932) have shown very clearly in *Zea Mays* that the nucleolus lies attached at the side of the achromatic thread of the satellite and not around it. In *Callisia* (Fig. 22, meiotic stage) and *Paeonia* (Fig. 29) this association seems to be between the satellite and nucleolus instead of between the achromatic thread and nucleolus.

A behavior of extrusion of the nucleolus into the cytoplasm in microsporogenous cells will be more fully described below. In Pinus somatic cells not a trace of the nucleolus was found at metaphase, as was also reported by Zirkle (1931); in *Paeonia* very rarely one or two small pieces were found just before mitosis (Fig. 29); in *Callisia* often there were found one or two pieces just before (Fig. 4) and after (Figs. 16 and 17) mitosis. A similar phenomenon was observed in Polystichum root-tip sections. Fig. 45 is a diagrammatic representation of the metaphase from side view. Outside the division sphere there is a nucleolar body that is a remnant from an earlier division stage. Similarly, in Fig. 46, is shown a large nucleolus outside the nucleus which is in prophase stage. Fig. 47 shows a nucleus with a typical nucleolus from an active region. The large nucleolus in the nucleus shows a bag-like feature as if containing a number of free bodies inside a bag, while the ones outside in the cytoplasm (Figs. 45 and 46) are uniformly round and homogeneous. Besides the large nucleolus shown in Fig. 46 there are a number of small nucleolar particles, staining red like the large ones, which may aggregate into various forms, as shown in Fig. 45, and finally be extruded into the cytoplasm, where they may further coalesce into a more compact form (Figs. 45 and 46). This peculiar form of nucleoli in such small granules may be comparable to the so-called amphi-nucleoli described by Wilson (1925). No speculations are justified here as to either their analogy or homology, since the present author has not made a study of this situation in animals. However, in *Polystichum* these particles show the same behavior as is characteristic of larger nucleolar bodies, indicating that they are of the same nature as the large ones. Extrusion of nucleolar material, in part or in whole, is reported by many investigators, among whom may be mentioned

Davis (1903) and Wakayama (1930), working with fungi, Yamaha and Sinoto (1925) on some thirty species and forms of phanerogamic plants, while in some Protista (Belar, 1928) the nucleolus does not disappear at all during mitosis but divides into two which are included in the sister nuclei.

In fixed material of *Callisia,* as well as in living, in the stigma hair nucleus there often were found around the region of the nucleolus some small extranucleolar bodies (Fig. 13). When subjected to differential stain, these bodies differed in their coloring when compared with the bright red of the nucleolus,.their color being of a darker shade of red. According to Heitz (1928), these bodies are considered to be chromosome fragments, but recently this assumption was refuted by Scheuber (1932), who finds that these bodies vary in number, size, and shape and show differences in staining when compared with the nucleolus. In *Callisia* the situation was found to be identical with that reported by Scheuber. When fixed material is studied carefully, one will generally find a clear area around the nucleolus, no matter how small it may be, while around these bodies no such clear area is observable, and their staining is intermediate, though more like nucleolus than chromosomes.

Similar bodies were observed in the microspore cells of *Callisia.* Fig. 27 represents a late prophase stage with six chromosomes, a large nucleolus in the middle, and a body at the side. This body can not be confused with a satellite, since the four short chromosomes point away from it and also because of its characteristic stainability referred to before. Fig. 28 is the drawing of a metaphase with such a body at the distal end of the satellite chromosome. Again it should be stated here that this body stained characteristically and was no doubt an extranucleolar body. It seems to have a remarkable stability in size and does not disappear as the nucleolus would. Identical bodies were observed during anaphase and telophase where it could be seen that they are left outside the new nucleus in the cytoplasm, there perhaps to disintegrate eventually. Such bodies were also found in root-tip cells of *Callisia.*

II. The Nucleolus in Microsporogenous Cells. Undoubtedly the process of nucleolar origin in the telophase of archespores is the same as was described for the somatic cells of root-tip material. No favorable stage having been found, this point could not be illustrated.

Figures 14-17 are from very young microsporogenous tissue of *Callisia.* At this stage quite commonly were found large extranuclear nucleolar bodies. In Fig. 15 there are two sister cells that have developed at a parallel rate, both being at late prophase stage. At the outside of each nucleus there is a large nucleolar body in an opposite

position to the corresponding one in the sister cell. Undoubtedly these two extranuclear nucleoli are remnants from a like cell shown in Fig. 14. Figs. 16 and 17 show the behavior of a nucleolus that has not been dissolved completely during the earlier stages of mitosis and is forced into the cytoplasm where it may remain for a considerable time during later stages of meiotic development, since these cells are destined to become microsporocytes. Fig. 18 represents the earliest stage of a microsporocyte after emerging from a mitotic division similar to the one shown in Figs. 16 and 17. In this, besides a large nucleolus being present among chromatic threads, one medium-sized nucleolus is found outside the nucleus in the cytoplasm probably resulting from a process illustrated in Figs. 15-17. In Fig. 23, which represents a much later stage, a nucleolus is found outside very near to the main nucleolus inside the nuclear membrane to which two chromosomes seem to be attached, while there is still another extra round body within the nucleus which is free from any such connection. Inside the cell represented in Fig. 21 there are present two such free round bodies, besides the larger nucleolus with a chromosome attached to it, that also show no connection with any chromosomes. In Yucca microspore mother cells there are either two medium-sized or one large nucleolus present inside the nucleus, as shown in Fig. 55. This figure is drawn at the nucleolus level, and not all the chromosomes are included in it. It must be emphasized here that the nucleolus is free from any chromosome attachment. The nucleolus in *Yucca* retains its size till the time of disappearance of the nuclear membrane, then it is extruded away from the division plane, sometimes one nucleolus to one side and another to the other side of the division plane, or both to one side individually, or two closely together, as shown in Fig. 56, there perhaps to disintegrate gradually. The nucleolar extrusion referred to in *Callisia* and *Yucca* may be analogous to a similar but seemingly more common behavior in *Polystichum*.

As is described above, the nucleoli that have not completely dissolved after the disappearance of the nuclear membrane are not retained in the newly-forming cells but are forced outside; so this behavior may be considered the primary rule but perhaps not the only rule. As is the case in some Protista described by Belar (1928), Christoff and Gentscheff (1932) have assumed that the nucleolus divides during mitosis, and the pieces of these nucleoli are retained in the newly-forming sister nuclei. So far most of the findings by many investigators and the present author indicate the contrary. However, in the present study there was encountered a difficulty which may be explained if it is assumed that rarely some of these pieces of nucleoli may be enclosed among the dividing chromosomes and retained in some of the nuclei.

As was pointed out, the nucleolar pieces shown outside the nucleus in Figs. 15 and 18 took a bright red color similar to the ones inside, while the free nucleoli inside the nucleus in Fig. 21, one outside and one inside the nucleus of Fig. 23, stained somewhat differently. If these free bodies are actually carried over from an earlier cell, the age of these nucleolus-like bodies may account for their reaction toward the stain, thus staining somewhat differently than the true nucleolus. They may remain there as inert particles. The above is presented as a mere suggestion, since the presence of these extra bodies is difficult to explain in the absence of experimental data. These bodies in the microsporocytes are of rare occurrence.

Another feature in *Callisia* was the presence of a small bud-like protuberance on the nucleolus, as shown in Fig. 22. A phenomenon similar to this is reported by Gates and Latter (1927), Maeda (1930), and Selim (1930). Gates and Latter find at the points of chromosome attachment to the nucleolus dark staining bodies, varying in number, size, and distribution. Maeda similarly finds small nucleolar bodies on the "mother nucleolus" and sometimes free from it in the nuclear cavity. Selim, studying meiosis in rice, finds the nucleolus budding off a "secondary nucleolus" at late diakinesis. He supports the view that the secondary nucleolus contributes material to the chromosomes, while the primary may contribute to the spindle.

Similarly, as in mitotic sister nuclei, in the sister nuclei of first meiotic division there were observed a number of small nucleolar globules (Fig. 26) which were found clearly oriented along the chromosome threads and not in the clear space of the nucleoplasm. Owing to some technical difficulties arising from poor fixation, no critical drawings of meiotic stages of *Paeonia* and *Pinus* could be presented. Only in an outline fashion the number and depth of nucleoli in *Pinus* (*P. Thunbergii*) from earliest to latest stages of meiosis are given (Figs. 39-44) to show the chromosome and nucleolus relationship. However, no radical differences from *Callisia* were observed in these plants. In *Paeonia suffruticosa* (greenhouse plant—other members of this genus were too young for the study of the meiotic phase of the problem) the nucleoli in the earliest stage numbered from six to one, while at later stages the number decreased to three to one, disappearing with the disappearance of the nuclear membrane. In *Pinus* at early leptotene (Fig. 39) and during early diplotene (Fig. 40), as many as nine nucleoli were found, the number decreasing as the development of the nucleus advances, but the volume of total nucleoli not showing any decrease. Fig. 41 is from late diplotene, while Fig. 42 is from late diakinesis stage when the nuclear membrane is disappearing or newly disappeared prior

to the chromosome arrangement in a metaphase plate. Fig. 43 is one of the sister cells after the first reduction division, while Fig. 44 is after homotypic division before the nuclei go into resting stage. In these two figures the number of nucleoli reached as high as nine to twelve.

III. Relationship of Nucleolus to Satellites and Secondary Constrictions. Heitz (1931a and 1931b) has made an extensive study of satellite and secondary constriction number in *Vicia* and other plants upon which he has based a definite theory concerning the origin of the nucleolus. His conclusion is that the number of nucleoli in any species in telophase sister nuclei should correspond to the satellites and secondary constrictions there present, for he assumes and gives evidence, as was mentioned above, that each nucleolus at telophase originates on and around the achromatic thread of each satellite or secondary constriction in the form of a collar. This correlation could be more readily proved if there were never found more nucleoli than satellites and secondary constrictions characteristic of a species. However, it is significant that the number of nucleoli in the greenhouse plant of *Paeonia suffruticosa* never reached the highest number found in the Arboretum plant of the same species. In the former the highest number was found to be five (Fig. 34), while in the latter seven, which have respectively three and four satellites. Both *P. Woodwardii* and *P. Delavayi alba* have six satellites; in the former the number of nucleoli reached as high as nine (Fig. 35), while in the latter quite rarely to as high as eleven (Fig. 36). Similarly, in *Callisia* quite rarely there were found three nucleoli (Fig. 12) instead of two to correspond with the number of satellites; in *Pinus* (*P. Strobus* root-tip) the number reached as high as fourteen (Fig. 38) where there should have been only ten if there are ten achromatic areas in the twelve pairs of chromosomes. There can be no doubt that a discrepancy to Heitz's expectation exists, the cause of which should be looked for elsewhere. However, the above data clearly indicate that whenever there is an increase of satellites or secondary constrictions, there is a similar increase in the number of nucleoli. The analysis of the situation is given below.

IV. Nucleolar Symmetry in Sister Nuclei. Before I had occasion to review the literature on the nucleolus, the perfect symmetry in some sister nuclei in root-tip sections, as illustrated in Fig. 33, had impressed me, and I was led to assume that this symmetry was due to the spatial relationship existing between the chromosomes in the sister nuclei. Therefore the mirror image of some nuclei in having nucleoli similar in number, size, and position was thought to be primarily due to the proportionality of the spaces between the chromosomes of the

sister nuclei; hence the symmetry of nucleoli in these cells. However, further work on this phase made it necessary to alter somewhat this original assumption.

Fig. 33 is a somewhat diagrammatic representation of the symmetry between four nucleoli in two sister nuclei. The symmetry is not only in number but also in size and position of the nucleoli. This drawing is from a root-tip section of *Paeonia suffruticosa* with the four satellites (as illustrated in Fig. 50). According to Heitz, the size of a nucleolus depends on the length of the satellite achromatic thread and its position on that of the satellite. In Fig. 33 the four nucleoli show a symmetry of number, size, and position of nucleoli but not a symmetry of the type expected according to Heitz, since in this plant two satellites are proximal and two are distal to the division poles (Fig. 50), while here one nucleolus is proximal and three are distal. There is also a size difference; the polar nucleolus in each nucleus seems to be more than the total volume of the other three nucleoli. Heitz would explain this on the basis that the increase of a nucleolus is proportional to the space around it; however, there were found many cases, as illustrated in Fig. 34, where there are small nucleoli which seem to have ample space to draw material and grow in size if Heitz's assumption is correct, but nevertheless they have remained small.

De Semet (1913) has proposed a genetic relationship between certain chromosomes and the nucleoli on the theory that nucleoli originate from certain chromosomes, and owing to this relationship he assumed the existence of a symmetrical relationship between sister nuclei. Yeates (1925) and Sprumont (1928) have supported de Semet's view, while Abele (1930) has explained this symmetry by assuming that "Die Nukleolar-substanz wird bei der Karyokinese in gleichen Mengen auf beide Tochterzellen verteilt, infolgedessen sind die Nukleolen oder Nukleolensatz beider Tochterkerne gleich gross."

It was pointed out earlier that the number of nucleoli during the early stages of nuclear development corresponded quite closely to the number of satellites present for the species in question, although quite often there were found more nucleoli than were expected according to Heitz's theory. However, since there seems to be some correlation between the number of satellites and the number of nucleoli, and also since during meiosis the nucleolus is constantly attached to a satellite chromosome pair in *Callisia* (Figs. 21-25), (Sax, 1932), in *Ranunculus* (Sorokin, 1929), and in *Zea Mays* (McClintock, 1931 and Burnham, 1932), therefore the symmetry often found between two sister nuclei in the number, size, and position of nucleoli is suggested to be primarily due to a physical relationship between a satellite and a nucleolus, and

independently to corresponding spaces between the same set of chromosomes in the sister nuclei, where nucleolar globules "exuded" from the adjacent chromosome surfaces fill in. The decrease in number and increase in size of the nucleoli may come about from the increase in these interchromosomal spaces during the thinning of the chromosome threads and increase in nuclear volume as a whole, allowing these spaces to fuse into fewer and larger spaces, followed closely by the fusion of smaller nucleoli into fewer and larger ones.

V. The Number and Size of Nucleoli in Polyploid Races. De Mol (1926 and 1928) has made some assumptions concerning the size and number of the nucleoli in the di-, tri- and tetraploid varieties of Hyacinths and has concluded that the size of "complex nucleoli" and the number of "simple nucleoli" are proportional to the number of the chromosomes. He believes that after nucleolar globules have been formed during telophase, they fuse into a "complex nucleolus" which later fragments into two, three, or four "simple nucleoli," depending upon whether the variety is diploid, triploid, or tetraploid. The present author has observed the process of fusion between two nucleoli at prophase in living tissue but has never observed fragmentation. If fragmentation ever occurs, certainly it must be in very rare circumstances instead of being the rule. The abundance of higher number of nucleoli in early stages of nuclear development and lower number in later stages must mean one thing only, that this decrease of number and increase in volume of nucleoli come about through fusion of higher number and small sizes into lower number and larger sizes. Here may be reported the results of my own observations from diploid, triploid, tetraploid, and pentaploid *Petunia*.

The largest single nucleoli from longitudinal sections of root-tips of six plants (Table 1) were measured in order to establish the size relationship of nucleoli in the polyploid series. The first two of these plants were diploids (7Lx and $7Lnc_{2-8}$), the third a triploid ($14S_{2-28} \times 7Lnc_{9}$)-1, the fourth and fifth tetraploids ($14S_{2-29}$ and $14L_{2-21}$) and the last a pentaploid (Trip.4 $\times$ $14S_{2-29}$). For the description of these plants the reader is referred to the author's paper on "Polyploidy in Petunia," 1931. On the slide of some of these plants there were either two or three root-tips in serial sections; in that case ten measurements were taken from each root, one from each serial section. It must be remembered that on one section there are hundreds of cells and as many or more nucleoli; therefore, even if only one measurement is taken from a section, it is from one of the largest among hundreds of nucleoli. During the measurements it was found that there was

some constancy in the size of the largest nucleolus for all the sections of the same root; therefore, taking measurements from more sections of the same root-tip was not considered essential. Further, in order to make these measurements more proportionate and uniform, only the largest and circular appearing nucleoli were chosen to avoid confusion and error in the measurements. Below, in Table 1, are given the diametric measurements of nucleoli in microns.

It is apparent from Table 1 that no significant conclusion can be arrived at as to the increase in volume of nucleoli from the increase of chromosomes of diploid to polyploid series, perhaps for the following reasons: (1) the number of measurements appears to be small and (2) the races tabulated here are of mixed nature. At present, unfortunately, there are no available plants to make this study more extensive; therefore, of necessity, the above measurements had to be limited to the numbers presented in this table. Even though these numbers are perhaps small for an exact conclusion, it seems evident that in order to have a basis of comparison on this phase of the problem, one is required to choose plants of polyploid series that are of the same origin. Table 1 shows that even though the second diploid plant (7Lnc) is from a mutated bud from the first diploid (7L), the difference in their measurements is quite striking. The 4n plants and the 5n are derivatives from an entirely different race of diploid, while the 3n strain is from a cross of this 4n and the second 2n race. Perhaps in the case of 3n the small measurement in general may be explained by assuming that the second 2n plant had a diminution factor affecting at least the size of the nucleolus. The two tetraploids and the pentaploid measured practically the same and were somewhat smaller than the first diploid.

In the writer's paper on *Petunia* is discussed the origin of these polyploid strains. From an ordinary diploid race the writer obtained a tetraploid plant, and from these two strains were obtained some triploid strains, and finally a pentaploid strain was obtained by crossing a triploid with a tetraploid. From the old slides of these strains, cross sections, similar measurements of nucleoli were taken. The origin of all polyploid strains, as can be seen, is from a pure-breeding diploid plant; therefore, the measurements from these plants are expected to give more of the true picture concerning the differences in nucleolar volume (Table 2). In this case measurements were taken from ten serial sections of one root-tip from each plant.

Table 2 seems to indicate an increase in diameter of a half a micron from 2n to 3n, one micron from 3n to 4n, and a decrease of one micron from 4n to 5n. There seems to be no difference between the 3n and 5n strains. From these measurements, as from those in Table 1, it may be

Table 1

Petunia series			Nucleoli measurements in microns of ten sections from each root-tip																Range	Median
Strain	Pedigree	Root-tips	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0		
2n	7L$_2$	1st								2	8								5.4–7.0	6.2
		2d														3	2	5		
		3d											4	1	1	2	1	1		
2n	7Lnc$_{2-8}$	1st			1	1	1	4	2	1									4.0–5.4	4.7
		2d				5	2	1		2										
		3d		1	6	3														
3n	(14S$_{2-28}$ x 7Lnc9)-1	1st		2	2	4		1			1								4.2–5.8	5.0
		2d				1	1	1			4	2								
		3d			1	5	1	1		4										
4n	14S$_{2-29}$	1st									2	7	1						5.4–6	5.7
		2d									1	1	6	2						
4n	14L$_{2-21}$	1st										2	3	3	2				5.2–6.4	5.8
		2d							1		1	8								
5n	Trip.4 xPet.2-29	1st											2	6	1		1		5.4–6.8	6.1
		2d								4	5	1								
		3d									2	4	2	2						

difficult to conclude whether or not there is any increase or decrease in the volume of the nucleolus along with the increase of chromosome number from diploid to polyploid series, according to de Mol's theory. However, there may be a tendency toward slight increase in nucleolar volume, due to an increase in number of chromosomes, and also some other factors may be involved in influencing the volume, as in the case of the difference between the two diploid strains in Table 1 and the equality of volume in the triploid and pentaploid in Table 2.

Table II

Petunia strain	Nucleoli measurements in microns of ten sections of one root-tip from each strain														Range	Median
	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8		
2n	1	2	4	3											5.2–5.[illegible]	5.5
3n				2	3	2	3								5.8–6.4	6.1
4n						1	3		1	1			2	2	6.2–7.8	7.0
5n				4	4	2									5.8–6.2	6.0

E. THE BEHAVIOR OF THE NUCLEOLUS IN LIVING TISSUE

An intensive observation was made on Callisia stigma hairs, kept alive either in tap water or 5% sucrose solution, in order to have a basis for determining how the fixed material differed from, or how closely it resembled, the living with regard to the behavior of the nucleolus and its relationship with the chromosomes. Entire pistils or styles with stigma hairs were placed in a drop of water on a slide and covered with a cover glass, and the material was studied under low or high power magnification with oil-immersion objective. These hairs, a few scores in number to a stigma, are fine and non-septate cells reaching a length of three millimeters, and were found to be very favorable material.

I. The Area around the Nucleolus. The nuclei and nucleoli in the hairs are considerably larger than those in some other parts of the somatic tissue, such as the style cells, where they may be one-fifth as large. The nucleoli in these cells may measure 4 to 10 microns in diameter, small in the very young and large in the hairs just before the bud opens. With a slow change of form of the nucleus, the nucleolus also changes its position and shape, perhaps owing to pressure exerted by the chromosome threads of the ever moving nucleus in the streaming cytoplasm. There was never found a clear area (separation area) between the nuclear reticulum and the nucleolus. This area appeared whenever these hairs were subjected to a disturbance by introducing a

toxic fluid under the cover glass, resembling the area so commonly observed around the nucleolus, inside the nucleus, of fixed material. Based on this observation the conclusion may be drawn that the clear area around the nucleolus in the fixed material is due to fixation and therefore is an artifact. Fig. 13 represents, in an outline, a nucleus in which is shown the clear area around a vacuolated nucleolus. As a result of any slight injury, physical or chemical, the nucleolus, as if sensitized, immediately pulls itself to one side, somewhat shrunken, close to a globular extranucleolar body (if any is present). Simultaneously, similar shrinkage takes place in the nucleus, enlarging this clear area. The creation of this area seems to be primarily due to sensitizing of the protoplasm (used in a broad term) and secondly, if at all, to plasmolysis. This assumption is substantiated by the fact that, in some cases, when the hairs were treated with Lewitzky's fixative, a clear area was created immediately but very soon disappeared, and no appreciable area was left between the nucleolus and nuclear mass, while when a stronger reagent was used, such as Bouin's or Flemming's, the effect was permanent.

II. Extranucleolar Bodies. In the earlier part of this paper reference was made to the nature of the extranucleolar bodies in somatic cells in connection with their difference in stainability and their possible homology with extranucleolar bodies in meiotic cells. In the stigma hairs of *Callisia*, as illustrated in Fig. 13, these bodies are generally found close to the nucleolus. Whenever the hairs were subjected to toxic fluids, the nucleolus seemed always to pull itself away from the nuclear mass and to remain attached to one of these bodies like a balloon attached to a mast. No adequate explanation can be given at present as to the origin of these bodies, small in size and varying in number from one to five or more. Because of their being near the nucleolar region, they may be considered some sort of extruded material from the nucleolus, or perhaps pieces of nucleolar material that were carried and held among dividing chromosomes and were included inside the nucleus and retained there along with the nucleolus as inert pieces of nucleoli. The present author is inclined to consider the last assumption more likely than the first. In general there was only one body of this kind present in the nucleus, and often there were nuclei without it. It was said that they were always near the nucleolus, but never were any of these found fusing with the nucleolus, which may be considered indicative of their difference, although the nature of this difference is not known.

III. Chromosome and Nucleolar Relationship. One of the principal reasons for studying living tissues was to determine what rela-

tionship exists between the chromosomes and nucleolus. In the style where cells could be found in late prophase stages, the association between the chromosomes and nucleolus was identical with that illustrated in Fig. 1. Obviously the chromosomes are separate units, and many at least are free from any connection with the nucleolus. In the stigma hairs the nucleus appeared very finely granular and compact but was undoubtedly made up of fine threads which never thicken, perhaps because of the lack of cell division in these hairs. The hairs themselves grow in length as the pistil matures and dry up and die after they have played their part (if they have any) during anthesis. With the growth of the hairs there is an increase both in nucleus and nucleolus and a slight decrease (at least in nucleolus) when the hairs become exposed at anthesis.[1] These hairs were treated with fixative to bring about a clear area around the nucleolus to see if there was any close connection between these fine threads and the nucleolus. The only apparent connection which could be found was that between the extranucleolar body and the nucleolus; and sometimes, in addition to some achromatic threads, there were very rarely found threads crossing this clear area, apparently being dragged by the nucleolus, owing to the separation of the nuclear mass and the nucleolus. Therefore it seems that even at early stages there is no complete direct association between all the chromosomes and the nucleolus and that a single attachment point between a chromosome and a nucleolus can not be considered of any physiological importance.

IV. The Nucleolar Vacuoles. Contrary to Fikry's belief (1930) that vacuoles in the nucleoli are artifacts, the present author found that vacuolation was one of the most important features observed in living as well as in fixed material. At times the nucleoli in all the stigma hairs were found to be vacuolated; at other times only part of them were vacuolated; and at still other times there were stigmas with hairs none of which contained vacuolated nucleoli. It is apparent that vacuoles are not permanent features (in the strict sense of the word) of the nucleoli, but vacuolation may be considered a normal phenomenon, and

[1]As a record I should like to mention that in the case of *Callisia* the size of the nucleolus is not constant all through the plant system. In microsporogenous tissue it is constant in all cells, at all stages, measuring about 4 microns; in the stigma hairs it varies from 4 to 10 microns, depending upon the stage of growth of these hairs; in the tissue of style and stamen filaments it measures about 2 microns; while in root-tips, in regions where there is active division, the size may be about 4 microns, and in the epidermal and root-cap cells about 2 microns. In all these cases there is a correlation between size of nucleolus and nucleus. The differences here recorded may be due to difference of nutrition, so that whenever the supply of food is less, there is a corresponding diminution in the size of both nucleus and nucleolus and vice versa.

vacuoles may appear and disappear normally. To check up this assumption, stigma hairs in water cultures were kept as long as they could be kept alive to see if non-vacuolated nucleoli would become vacuolated.

For this experiment young pistils or styles with stigma hairs cut off from pistils were put in a drop of water on a slide and covered with a cover glass. They were first studied under the microscope with oil-immersion objective, the desired regions spotted, the hairs and nucleolus in its surroundings sketched, and the nucleolus measured if necessary; then the slides were put away in a petri dish. At the bottom of the petri dish was placed some wet filter paper to create a moist chamber. This method enabled me to keep some of this material alive in tap water for six days. After many trials not a single unvacuolated nucleolus had become vacuolated. Some appeared to contain vacuoles in the form of small droplets, but this proved to be a sign of degeneration which was followed by the death of the cell.

The general effect of this treatment was that in the living cells the nucleoli always showed considerable decrease in volume. One nucleolus was measured soon after the material was put on the slide. It measured 9 microns in diameter; at the end of the second day it had decreased to 6 microns, to 4 microns at the end of the third day, and to 1.8 microns at the end of the sixth day; and finally the cell died. Results of this kind would lead one to suppose that the nucleolus was used up as reserve food by the "starved" cell and hence its extreme decrease in size. However, a similar decrease was noticed in the nucleus itself but not in the same proportion (no measurements of nuclei were taken).

Analogous behavior is reported by Meyer (Guillermond et al., pp. 183-184, 1933), who believes that the nucleolus is made up of "ergastique" elements of the cell and that the volume of the nucleoli is essentially variable during the physiological state of the cell. The volume of the nucleolus increases when the cell has an abundant supply of nutritive material and diminishes when the cell is in a starving condition. In the mesophyll of *Galtonia candicans* leaves Meyer finds that there is a diminution in the volume of the nucleolus when these leaves are etiolated and states: "Si, dans la feuille vivant dans des conditions normales, on exprime ce volume par l'unite, on constate qu'il s'est abaissé à 0,38 dans une feuille maintenue à l'obscurité pendant 36 jours et à 0,18 dans une feuille maintenue à l'obscurité pendant deux mois." During the change of the albumen of *Galtonia* this author finds some interesting changes in nucleolar volume which are described thus: "dans les cellules de l'albumen jeune, les nucléoles mesurent 52,3 environ; leur volume s'accroît à mesure que l'albumen se charge de matériaux

nourriciers, jusqu'au volume de 101,3; puis il diminue au moment de la formation des parois cellulaires, et surtout au moment de la germination et de l'utilisation de réserves, jusqu'à devenir insignifiant."

To supply food matter for the cells to prevent them from "starving," some material was kept in 5% sucrose solution in vials and on slides. In this solution it was possible to keep these hairs alive for eighteen days. At the end of this time, owing to the growth of yeast cells and molds of various kinds, the tissue as a whole was destroyed. The striking difference between water and sugar cultures was that while there was invariably a decrease of nucleolus in the water, there was hardly any appreciable decrease in the sugar solution, except that whenever the water under the cover glass had evaporated in part, the nucleoli were found rounded up and the flow of the cytoplasm slowed down. As soon as more water was added again, the cytoplasm increased its speed of flow, and the nucleoli took on ellipsoid or other shapes due probably to the activation of the nucleus. In one case one cell was recorded dead because there could not be observed any movement in the cytoplasm, but when water was added, it soon revived and showed cytoplasmic streaming.

It was observed that the vacuolated nucleoli did not all lose the vacuole when they were kept in sucrose solution, except in a few cells, indicating that the tissue can live normally in the sucrose solution, for a while at least, while if kept in tap water, some radical change (superficially physical) seems to take place. During these experiments, however few in number, no vacuolation, *de novo*, was observed even in the sugar solution. This was perhaps due to the somewhat abnormal condition in the sugar solution (if it is assumed that vacuolation is a normal phenomenon), since this solution can not be considered an ideal medium for tissue culture.

Some measurements were taken of nucleoli, some of which were vacuolated and some not. In all cases there was a decrease in volume in water cultures irrespective of the vacuole. Exact measurements were taken of two nucleoli, one with a vacuole and the other without. For convenience, let the vacuolated be No. 1, and the nonvacuolated No. 2. The diameter of No. 1 was 6.2 microns and that of its vacuole 5 microns. The diameter of No. 2 was also 6.2 microns. Overnight, sixteen and a half hours, the vacuole of No. 1 had disappeared, and the nucleolus had decreased in size to a diameter of 5.4 microns. The diameter of No. 2 also had decreased to 5.6 microns. The measurements after 26 hours were, for No. 1, 4 microns, and for No. 2, 4.2 microns. After some hours both cells were dead. As can be seen from these measurements, the disappearance of a vacuole does not seem to have any appre-

ciable effect on the volume of the nucleolus. Therefore, it is perhaps safe to assume that the vacuolated region has been transformed into true nucleolar substance by some physiological phenomenon, the exact nature of which it is difficult to ascertain. This region appears to be of the same concentration as the cell sap. This assumption is based on the fact that in one case a small proplastid-like body was found inside this region and showed the same rate of Brownian movement as took place outside the nucleus.

F. GENERAL DISCUSSION AND CONCLUSIONS

I. Chromosome Morphology and Chromosome-nucleolar Relationship. Conclusive evidence is presented in the text to show that the chromosomes in somatic nuclei keep a form of linearity which remains practically constant from anaphase to the end of prophase; that there exists, in general, a permanence in position and structural form during these same stages. The facts seem to indicate that at no time of development do the chromosomes pass a period during which time they are in a granular state and thrown about in a haphazard way, later to reestablish themselves in threads, joining end to end, again to break apart into separate units as chromosomes.

There is no doubt, among those familiar with karyokinetic problems, of the individuality of chromosomes in structure. The truth of this matter seems to have been substantiated by Rable as early as 1885 and by Boveri (1909) and, more recently, by Kagawa (1926), Belar (1929), Koerperich (1930), and others. Rau (1930) states that in *Cyanotis cristata* pollen-mother-cells no continuous spireme appears to be formed at any stage. It is a fact that generally, morphologically, the chromosomes of a basic set in a species differ considerably from each other; therefore the possibility of diverse chromosomes, such as described for *Callisia* and *Paeonia*, forming a continual spireme is inconceivable. The conclusion may be that, normally, the chromosomes never join end to end. This point may be further emphasized by referring to the work of Sax and Anderson (1933) and others who show that during meiosis some chromosomes may interlock, a phenomenon which could not take place if these chromosomes were parts of the same continuous spireme thread. These facts indicate that the direct flow of nucleolar material into and through all the chromosomes, as suggested by some investigators, is an impossibility.

II. Origin and Development of the Nucleolus. It is shown that the nucleolus originates in the form of small globules on the surface of the chromosome threads during the late telophase stage, that

very soon there is a close grouping of chromosomes, and that a demarcation between the chromosomes as a group and the outside cell-sap and cytoplasm is established. It is difficult to show if there is a definite nuclear membrane, as such, around the chromosome group at this period, but for practical purposes it may be assumed that such a membrane exists from the beginning of the late telophase till the time of metaphase plate formation. The method of origin of the nucleolus here reported was first described, I believe, by Van Camp (1924).

The process of nucleolar development appears to be a surface phenomenon which can be explained by assuming that a chemical reaction takes place between the substance on the surface region of the chromosomes and the nuclear sap. The nucleolus, then, may be considered a by-product of the chromosome matrix and nuclear sap and, in this sense, may be identified with the matrix, as was suggested by Marshak (1931), but with this difference, that it is the matrix which produces the nucleolar substance by going into a chemical combination with some substance in the nuclear sap. The difference is indicated by their reaction to the differential stain. From this it may be deduced that there exists a striking difference between the nuclear sap inside a nuclear membrane and the cell-sap outside this membrane, because of the fact that the nucleolus will originate and develop inside a nuclear membrane and float in the nuclear sap, while outside this membrane the nucleolus disappears and apparently is dissolved in the cell-sap. It appears, therefore, that the cell-sap has a dissolving effect on the nucleolus, the rapidity of which may differ in different species; hence the lagging of nucleolar particles in some species and the lack of it in others which may be assumed to depend on the varying strength of reaction of cell-sap in different species.

The phenomenon of lagging may also be explained on the basis that there exists a difference in rate of chromosome development during the late prophase and metaphase, varying in different species. For instance, it was noticed that the chromosomes in *Callisia* (somatic stage) are well developed by the time they are forced onto the metaphase plate, already well split, and ready to divide and move toward the division poles. In *Paeonia* and, to a lesser extent in *Pinus*, this development is not so far advanced as in *Callisia* before the nuclear membrane disappears; the chromosomes of the former species stay at metaphase considerably longer, while in the latter the separation of sister chromosomes is much more rapid. It is suggested that into the phenomenon of lagging of nucleolar particles the time element may enter. Thus, in species where the chromosomes divide more rapidly at metaphase, as in *Callisia*, more nucleolar particles are observed during the metaphase

and anaphase; but rarely in *Paeonia* and never in *Pinus*, where the division of chromosomes is delayed, are these particles found.

Heitz recently has developed a theory to explain the origin of the nucleolus based on the fact that in sister nuclei there often exists a symmetry in nucleoli number, position, form, and size. He believes that there is a correlation between number and position of satellites and secondary constrictions and number and position of nucleoli in somatic cells, and that the nucleolus originates in the form of a collar around the achromatic thread that holds the satellite or secondary constriction segment to the end of the main chromosome body. Brunn (1932) indicates that *Primula seclusa* does not possess satellite chromosomes but possesses nucleoli. On the other hand, Geitler (1932) presents evidence supporting Heitz's theory by finding four nucleoli in telophase stages in a tetraploid form of *Crepis capillaris* which has four satellited chromosomes. It was mentioned in the text that the present author also found some such condition in the species where the satellite situation was thoroughly studied. For example, such correlation was particularly noticed in *Callisia* with two satellites with usually one or two nucleoli; in *Paeonia suffruticosa* in two individual plants with three and four satellites respectively with nucleoli constantly fewer in the former and more in the latter. However, it was found that this correlation was not complete, since it is decisively shown by Van Camp and by the present author that there is no localization of nucleolar development comparable to Heitz's assumption and that, even at resting stages, there are frequently found nuclei that contain nucleoli far above the number expected on the basis of Heitz's theory. It may therefore be pointed out that there are no definite nucleolus-producing chromosomes; that nucleoli may be produced in the form of small globules on the surface of every chromosome, which latter collect into larger globules; that some of these larger globules may come in contact with the satellites and remain attached there; and that finally the number of nucleolar globules may decrease to one by fusion between the globules, so that generally one large nucleolus is found by the time the chromosomes have developed to the late stage of prophase. De Mol's assumption of nucleolar fragmentation is found contrary to all observations made in all species reported here, and by no known mechanism can such a process be adequately explained; on the contrary, the present author observed in living tissue a fusion between two moderately large nucleolar bodies but has never seen any indication of fragmentation.

No adequate explanation can be offered for the origin of a protuberance (bud-like structure) at the side of the large nucleolus in meiotic stages nor for the extranucleolar bodies found both in somatic and

meiotic nuclei. Based on the staining reaction, it is suggested that the extranucleolar bodies may be undissolved nucleolar particles that have been carried by the dividing chromosomes into the new nuclei, there to remain as inert particles. It is difficult to see if there may be a relationship between the bud-like protuberance and extranucleolar bodies. Until the origin of both these bodies is carefully studied from living material, it will be futile to speculate as to their origin and function. Whenever there is a similarity of reaction of nucleolar bodies inside the same nuclear membrane, this may be explained by assuming that each represents a nucleolar globule which, owing perhaps to its being attached to a satellite, is not able to fuse with another such globule into a large single one. The similarity or dissimilarity between the true nucleolus and these bodies may be intelligible if a method of differential staining is applied.

III. Behavior and Function of the Nucleolus. The present author is inclined to put considerable stress on the behavior of vacuolation of the nucleoli and considers this phenomenon of significance, since it was pointed out above that a large vacuole may disappear completely and yet not cause any appreciable decrease in the volume of the nucleolus. Therefore it seems more accurate to assume that instead of the nucleolus being used up as reserve food, it is concerned in the general metabolic processes of the organism as a whole. This assumption may be more validated when we consider that the nucleolus is very probably universally found in all nuclei of all organisms. An exception to this rule is found in the male gamete, as cited by Wilson (1925), Sharp (1926), Tischler (1926), and Belar (1928). This exception is probably more apparent than real, however, for it seems likely that the nucleolus is not lacking but only delayed in its development. The chromosomes for a long time remain unmodified until the male gamete reaches the female gamete, and there they seem to go through a resting stage, during which time nucleoli in small globules come to appear. This conclusion is drawn from the figure presented by Wylie (1923) of *Vallisneria spiralis* and from the studies of Sax and Edmonds (1933) and O'Mara on *Lilium* (1933).

Montgomery (1898) makes the statement that the nucleolar vacuoles in *Spirogyra* are normal structures. It was found by the present author that the nature of these vacuoles does not indicate any peculiarity characteristic only of that species but a similarity in behavior comparable to nucleolar vacuoles observed in *Callisia* stigma hairs and in all other parts of the plant. The number of nucleoli varied from a few small ones, differing among themselves in size, to a single large one. It was

also noticed that thinly opened fine threads of the chromosomes surround the nucleolus, giving an appearance similar to the nucleus illustrated in Fig. 18. Conard (1931) seems to be justified in opposing some investigators who claim the origin of the chromosomes from the nucleolus. To this category may belong the findings of Kater (1928) and Faulkner (1929), who claim that nucleoli give rise directly to chromosomes.

In this connection some *Spirogyra* were treated with Ehrlich-Biondi stain which was introduced under the cover glass, and there was seen a distinct demarcation between the chromosome threads and the nucleolus. The chromosomes did not take any stain, while the nucleolus took a brick red color. It was also observed that the pyrenoids surrounded by the chloroplastic mass took a stain identical with that of the nucleolus. The similarity of staining reaction of the nucleolus and the pyrenoids may signify that they are composed of the same chemical material, or it may merely be a reaction due to their having the same electrical charge. The significance of their similarity in reaction with this stain will remain an open question until their chemical make-up is understood; therefore it is futile even to suggest that nucleoli and pyrenoids may have a homologous function, one in the nucleus and the other in the cytoplasm.

As mentioned in the introduction, according to de Mol (1926), Zirkle (1928), and Fikry (1930), the nucleolus may play a part in transmitting hereditary stimuli from the chromosomes to the cytoplasm. This theory may be justified from the fact that the nucleolus is built up from small globules that may either be exudation products from the chromosomes or formed on the surface of the chromosomes from a chemical combination between some material produced from the metamorphosing chromosomes during the late telophase or between matrix substance and material taken up from the nuclear-sap. It is shown that this "compound" product, the nucleolus, seems to stay unchanged in volume during the entire development of the nucleus, disappearing in the cytoplasm only after the nuclear membrane has vanished, or perhaps when it has gradually become freely permeable to cell-sap. The fact remains that the nucleolus disappears in the cell-sap either completely or partially, and in some cases, as in *Callisia* (Figs. 15-17), *Polystichum* (Fig. 46), and *Yucca* (Fig. 56), it may be left, in its entirety, in the cytoplasm and stay there undissolved, at least for a long time; while in other cases, as was indicated above, the nucleolus may not be dissolved in the cytoplasm at all, or may be included in the daughter nuclei, as seems to be the case in some Protista.

If the assumption is correct that the nucleolus is a compound product

originating, in part, from all the chromosomes and thus containing material from all the chromosomes, and hence from all the genes, and since, excepting perhaps in the case of some Protista, this substance is dissolved in the cell-sap during or after cell division, the suggestion of the above authors that the nucleolus may play a part in transmitting hereditary stimuli from the chromosomes to the cytoplasm deserves consideration. It may be assumed that the chromosomes fundamentally as self-perpetuating bodies divide up and are carried into, and become the basis of the daughter nuclei; that the chromosomes, as such, do not directly transmit gene stimuli, but that this transmission takes place, indirectly, through the matrix substance, which may be considered a by-product of the chromosomes. If this is correct, an explanation may be offered that would perhaps help in interpreting some data presented by Riley (1932) concerning the phenomenon of self-sterility in *Capsella*. The data of this author seem to indicate that a self-sterility factor, instead of exerting its inhibitory influence over only half the number of the pollen grains, as is the case in *Nicotiana* (East, 1929), affects all the pollen grains. In the case of *Nicotiana* the factors of self-sterility may not be so inhibitory as in *Capsella;* hence the difference in these two genera. Therefore a hereditary factor of self-sterility may be transmitted through the nucleolus into the cytoplasm of the microsporocyte and through the four pollen grains resulting from the division of the microsporocyte.

The presence of a bud-like protuberance on the nucleolus in rice meiotic cells has led Selim (1930) to assume that a single nucleolus buds off a secondary nucleolus which disappears during the development of the chromosomes and the primary one at late diakinesis. He supports the view that the secondary nucleolus contributes material to the chromosomes and the primary one to the spindle formation. Budding is taken by him as a separation of the nucleolus into two different materials. Sethi (1930) has entertained principally the same ideas as Selim. In general, the transportation theory seems to have many followers, based on the fact that there has been found some sort of direct connection between some chromosomes and the nucleolus, and also because there has been observed a difference in stainability of chromosomes in early and late stages, a faint stain, or none, being taken in early stages and a deep stain at late stages. With this is associated the disappearance of the nucleolus by the time the chromosomes are arranged on a metaphase plate. Harper, as early as 1905, working on certain mildews, refuted the presence of direct contact between the chromosomes and nuleolus. Fikry (1930), working with *Rumex,* suggests that the connection observed between nucleolus and spireme is

due to chance, and that there is no constant and fixed connection between them. The present author found no connection between the nucleolus and chromosomes in *Yucca* (Fig. 55); in *Callisia* and others the connection is only between certain chromosomes and the nucleolus.

It was asserted above that the diminution or disappearance of the nucleolus had no correlation either with the stainability of chromosomes or with building up of the chromosome mass. For this adequate explanations were offered.

The theory of the formation of the spindle by one of these nucleoli must be refuted because many investigators have observed and reported that nucleoli may be in part or completely extruded from the cytoplasm, but the chromosomes nevertheless divide, as illustrated in Fig. 56, without the assistance of the nucleolus and move toward opposite poles.

In this connection should be discussed the question of the electromagnetic quality of the nucleolus and chromosomes proposed in the following words by Zirkle (1928) as an addition to a number of theories proposed earlier by others: "The plastin, being electro-positive, changes the electro-negative spireme by flowing into it, to an electro-positive chromatin complex; thus the chromatin, which had collected at the equatorial plate as far as possible from the poles of the spindle, reverses its motion with its electrical charge and migrates to the two poles." Christoff and Gentscheff (1932) have subjected Zirkle's proposed point to a test by putting an electrical charge through a maize sprout, and find that nucleoli do show a definite charge, thus giving support to the above idea. However, it was mentioned early that in living material the present author has observed two large bodies of nucleoli fused together into one, that the nucleolus may be bodily extruded into the cytoplasm without an appreciable decrease in size, as is shown for *Yucca* in Fig. 56. Therefore, based on these two facts alone, the polarity phenomenon can not be responsible for dividing the chromosomes, because two similarly charged bodies ordinarily could not fuse into one, but would repel each other, and because in *Yucca* at least, the nucleoli being extruded into the cytoplasm, they are eliminated from playing any part in the division phenomenon.

It was stated above that the present writer is inclined to put considerable stress on the behavior of vacuolation of the nucleoli. It is very likely that the nucleolus may play a part in the organization of the cell as an organ that may primarily be concerned with assimilative processes by "imbibing" substances from the surrounding medium, converting them into material that may be utilized by the chromosomes especially for their growth and perpetuation. Schaede (1929) states

that "Directe Verbindung zwischen Kernfaden und Nucleolus in dem Sinne, dass ersterer in amöboide Fortsätze des letzteren einmünde, besteht nicht, auch kein unmittelbarer Übergang von Nucleolarsubstanz in den Kernfaden," and that "In Anbetracht ihrer gegenseitigen Beziehungen ist eine Abgabe von Substanz in abgeänderter Form aus dem Nucleolus in den Kernfaden wahrscheinlich . . ." As Schaede points out, the nucleolar substance itself can not be directly involved in this process, as Van Camp and many others have believed. It was amply shown that the nucleolar body remains undiminished during the chromosome development. Its assistance in this process may be of an indirect nature during the vacuolation and devacuolation described above. The latter phenomenon may not be abrupt but simultaneous, since no appreciable decrease or increase was observed during this process.

G. SUMMARY

1. Because of the evidence offered that the chromosomes never lose their individuality in structure and position inside the nuclear membrane, it is argued that a direct flow of any nucleolar substance from one end of a chromosome through the entire spireme is not possible. In the case of *Callisia* some, but not all, chromosomes may be attached to a nucleolus. Applying a delicate differential stain (Ehrlich-Biondi), there was never found an intermediate staining at any point of any chromosome that may have come in contact with the nucleolus, and at all stages the chromosomes were stained pale blue and the nucleolus bright red, indicating that no direct interchange of material had taken place between chromosomes and nucleolus.

2. No correlation was observed between a decrease of nucleolar volume and the process of "emerging" chromosomes. The volume of nucleolus in meiosis studied in some species, once the maximum is reached during late telophase-early leptotene, remains constant all through the chromosome development.

3. The satellite and secondary constriction phenomena in *Callisia, Paeonia,* and *Pinus* are analyzed to determine the nature of satellite-nucleolar relationship. The nucleolus is found to originate on the surface of the chromosomes during telophase in the form of small globules, as Van Camp has shown, and not on a specific region (achromatic threads of satellite and secondary constriction) of satellite chromosomes, as Heitz believes; hence there were found numerous globules instead of a limited number corresponding to the number of satellites or segmented chromosomes. When there was a connection between a satellite and a nucleolus, it was found that the nucleolus is attached at

the side of the satellite instead of forming a collar around the satellite achromatic thread.

4. There was found some correlation between the number of satellites and nucleoli, but not exactly as was expected according to Heitz's theory. The symmetry between two sister nuclei in the number, size, and position of nucleoli is suggested to be due primarily to a physical relationship between a satellite and a nucleolus, and to corresponding spaces between the same set of chromosomes in the sister nuclei where nucleolar globules "exuded" from the adjacent chromosome surfaces fill in.

5. A behavior of extrusion of nucleolar particles in the species studied is described. In *Callisia* there were found small extranucleolar bodies which showed a difference in stainability when compared with the nucleolus. Their origin could not be determined. It is suggested that they may be inert nucleolar particles representing pieces of nucleoli that have been retained in the sister nuclei as lagging particles. On the side of the meiotic nucleolus there was often observed a protuberance, a bud-like growth. Its nature and relationship, if any, with some of the free nucleolar bodies observed in meiotic nuclei could not be determined.

6. A critical analysis is presented of de Mol's finding that the size and number of nucleoli vary proportionately as the number of chromosomes is increased in polyploid series. No significant correlation was found in a similar relationship studied in *Petunia*.

7. The results of some observations made from living tissue (stigma hairs of *Callisia*) are as follows: (a) A clear area around a nucleolus is an artifact which can be produced by applying toxic fluids to stigma hairs in culture. (b) Small extranucleolar bodies were often observed in the nuclei of the hairs. The nucleolus was found showing some affinity to these bodies when a clear area was artificially created. (c) No general chromosome and nucleolar connections were observed. The connections, when present, may be limited to certain chromosomes. Often specific connection was found between the nucleolus and the extranucleolar bodies. (d) The vacuole in the nucleolus is normal and not an artifact and probably appears and disappears normally. The phenomenon suggestive of vacuolation is considered by the present author to be of physiological significance. Some data are presented concerning this behavior.

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EXPLANATION OF PLATES 64 TO 67

All sections are longitudinally cut.

Figs. 1–28 *Callisia repens*

Fig. 1. Method: Lewitzky fixation; stained in iron-alum-haematoxylin. Stage: prophase, oblique view, nucleolus in center, one probable attachment between nucleolus and chromosome.

Fig. 2. Method: same as in Fig. 1. Stage: prophase, side view (nucleolus not shown).

Fig. 3. Method: Lewitzky + 0.25% saponin; stained in i.a.h. first; destained and restained in Ehrlich-Biondi mixture. Stage: prophase drawn on nucleolar level, chromosomes stained homogeneously pale blue and nucleolus bright red.

Fig. 4. Method: same as in Fig. 3. Stage: metaphase with two nucleolar pieces.

Fig. 5. Method: same as in Fig. 3, before destaining. Stage: metaphase, the satellite chromosome pair, fully split; one of the sister chromosomes on the left shows a further split.

Fig. 6. Method: same as in Fig. 3. Stage: late anaphase, chromosomes homogeneously pale blue.

Fig. 7. Method: same as in Fig. 5. Stage: an anaphase chromosome with split and twist.

Fig. 8. Method: Lewitzky fixation; stained in crystal-violet-iodine. Stage: split and twisted chromosomes from late anaphase.

Fig. 9. Method: same as in Fig. 3. Stage: telophase; diagrammatic presentation of nucleolar globules.

Fig. 10. Method: same as in Fig. 3. Stage: telophase; two small nucleoli shown.

Fig. 11. Method: same as in Fig. 3. Stage: telophase, with one nucleolus larger than in Fig. 10.

Fig. 12. Method: same as in Fig. 1. Stage: interphase, with three nucleoli (the nucleoli were tested with E.-B. stain; all took bright red color).

Fig. 13. Method: same as in Fig. 1. Stage: dormant stage nucleus in stigma hair, a vacuolated nucleolus with clear area around and four extranucleolar bodies.

Fig. 14. Method: 1-3 minutes Carnoy solution, followed by Lewitzky fixative; stained in c.v.i., destained and restained in E.-B. Stage: prophase from a cell of microsporogenous tissue with two nucleoli drawn from the level of the nucleoli.

Fig. 15. Method: same as in Fig. 14. Stage: prophase of two microsporocyte sister cells, each with an extranuclear nucleolus, drawn from the level of the nucleoli.

Figs. 16 and 17. Method: same as in Fig. 14. Stage: late anaphase of a microsporogenous cell division, with median and polar lagging nucleoli, respectively.

Fig. 18. Method: Fixative same as in Fig. 14; stained in i.a.h. Stage: early leptotene, with a normal nucleolus inside the nucleus and one lagging nucleolus outside.

Fig. 19. Method: same as in Fig. 14. Stage: leptotene, with two small normal nucleoli, one with a small vacuole.

Fig. 20. Method: same as in Fig. 14. Stage: leptotene, with a large normal nucleolus.

Fig. 21. Method: 1-3 minutes Carnoy, followed by Zirkle (1928) No. 4; stained in i.a.h.; later destained and restained in E.-B. Stage: pachytene, one large normal nucleolus with chromosome attach-

ment and two extra smaller nucleoli which stained somewhat differently. (Some of these nucleoli were in the form of a bubble with a very thin membrane stained a somewhat lighter color; hyaline inside.)

Fig. 22. Method: same as in Fig. 21. Stage: pachytene, with a bud-like protuberance at the side of the nucleolus; a satellite chromosome attached to the nucleolus.

Fig. 23. Method: same as in Fig. 21. Stage: Pachytene, with one outside and one inside additional nucleoli, which are of the same nature as those indicated in Fig. 21.

Fig. 24. Method: 1-3 minutes Carnoy, followed by Lewitzky; stained in i.a.h. Stage: pachytene; nucleolus with four small vacuoles, dark stain in the nucleolar substance, somewhat hyaline in the marginal portion.

Fig. 25. Method: Lewitzky fixation; stained in i.a.h. Stage: diakinesis; at the side of the nucleolus a satellite (?) attached. (Chromosome pair shown in outline.)

Fig. 26. Method: same as in Fig. 25. Stage: interphase of first meiotic division, showing globular nucleoli. (Chromosomes could not be shown because of their very faint color; however, these globules were very closely associated with the chromosome threads.)

Fig. 27. Method: same as in Fig. 25. Stage: microspore prophase with a large nucleolus with one probable chromosome attachment, and an extranucleolar body below a long doubled-up chromosome.

Fig. 28. Method: same as in Fig. 25. Stage: microspore metaphase, with one satellite chromosome and an extranucleolar body near the distal end of the same.

Figs. 29 and 30 *Paeonia suffruticosa* (Arboretum plant)

Fig. 29. Method: Lewitzky fixation; stained in c.v.i. Stage: metaphase; a part of a satellite chromosome to which a small piece of nucleolus is attached.

Fig. 30. Method: same as in Fig. 29. Stage: anaphase; parts of chromosomes which are split and show spiral twists.

Fig. 31 *Paeonia suffruticosa* (greenhouse plant)

Fig. 31. Method: same as in Fig. 3. Stage: telophase, where small bright red globular nucleoli were seen along pale blue chromosome threads.

Figs. 32 and 33 *Paeonia suffruticosa* (Arboretum plant)

Fig. 32. Method: same as in Fig. 29, but destained and restained in E.-B. Stage: telophase, sister nuclei. Small bright red globular nucleoli are presented in a diagrammatic way to show approximate number of these globules.

Fig. 33. Method: same as in Fig. 29. Stage: late telophase, showing a symmetry in two sister nuclei in the number, position, and size of nucleoli. Fine chromosome threads are shown diagrammatically arranged in linear fashion. Four-satellited plant with four nucleoli. The number of nucleoli may be as high as seven and possibly more.

Fig. 34 *Paeonia suffruticosa* (greenhouse plant)

Fig. 34. Method: same as in Fig. 3. Stage: interphase of sister cells, each nucleus of which possesses five nucleoli; no symmetry of size and position. Three-satellited plant with five nucleoli.

Fig. 35 *Paeonia Woodwardii*

Fig. 35. Method: Lewitzky fixation; stained in E.-B. Stage: interphase. Six-satellited plant with nine nucleoli.

Fig. 36 *Paeonia Delavayi alba*

Fig. 36. Method and stage: same as in Fig. 35. Six-satellited plant with number of nucleoli as high as eleven, generally less than this number.

Figs. 37 and 38 *Pinus Strobus*

Fig. 37. Method: Lewitzky fixation; stained in E.-B. Stage: telophase; sister nuclei with numerous small nucleolar globules.

Fig. 38. Method: same as in Fig. 37. Stage: interphase; only nucleoli shown. Ten-secondary-constricted plant with as high as fourteen nucleoli.

Figs. 39-44 *Pinus Thunbergii*

Fig. 39. Method: Lewitzky fixation; stained in E.-B. Stage: early leptotene with nine nucleoli.

Fig. 40. Method: Lewitzky fixation; stained in E.-B. Stage: early diplotene with nine nucleoli.

Fig. 41. Method: same as in Fig. 40. Stage: late diplotene with six nucleoli.

Fig. 42. Method: same as in Fig. 40. Stage: beginning of metaphase with four nucleoli.

Fig. 43. Method: fixation same as in Fig. 40, but stained in c.v.i Stage: interphase after meiotic division. Only one of the two sister nuclei shown possesses nine nucleoli.

Fig. 44. Method: same as in Fig. 43. Stage: late telophase of tetrad formation, number of nucleoli varying from eight to twelve.

Figs. 45-47 *Polystichum acrostichoides*

Fig. 45. Method: Lewitzky fixation; stained in E.-B. Stage: metaphase, with small nucleolar globules in the division region, and one outside this region, remnant of an earlier cell division.

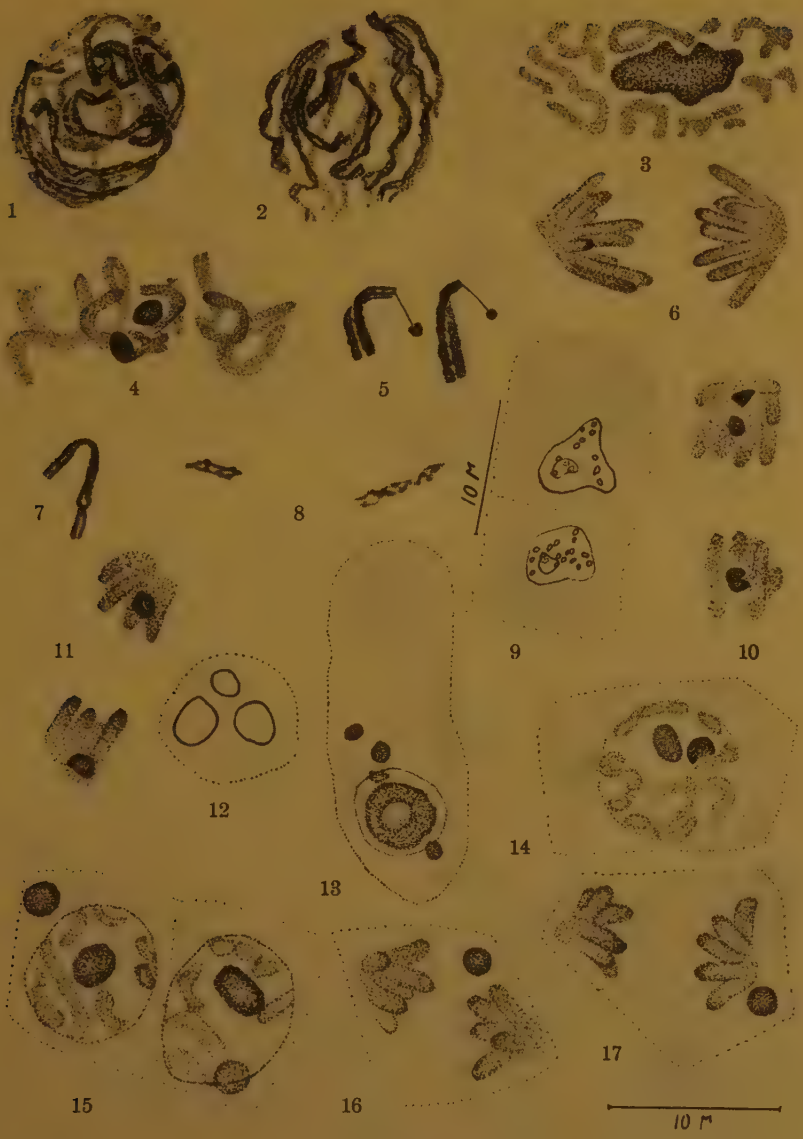
Fig. 46. Method: same as in Fig. 45. Stage: interphase from periblem marginal region with a large nucleolus inside, bag-like, containing darker staining particles; similar kinds free from the "bag." One large round nucleolar body stained darker like the small ones outside the nucleus, this body being a remnant of an earlier cell division.

Fig. 47. Method: same as in Fig. 45. Stage: a nucleus in early prophase with a single bag-like nucleolus containing darker staining smaller particles. This figure is from a more actively dividing region.

Figs. 48 and 49 *Callisia repens*

Fig. 48. Method: Lewitzky fixation + 0.25% saponin; stained in i.a.h. Stage: metaphase plate showing the structural differences of the chromosomes.

Fig. 49. Method: Navashin fixation, half strength; stained in c.v.i. Stage: anaphase.



Origin and behavior of the Nucleolus in Plants

THE MELIOTYPE CORP. BOSTON

ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

THE HELIOTYPE CORP. BOSTON

ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

THE HELIOTYPE CORP. BOSTON

SOME HYMENOMYCETES FORMING MYCORRHIZAE WITH PINUS STROBUS L.

A. B. HATCH AND C. TALBOTT HATCH

With plates 68 to 71

THE CLASSICAL EXPERIMENTS of Elias Melin (1922) yielded the first conclusive evidence that the Hymenomycetes are involved in the mycorrhizal associations of forest trees. The only fully satisfactory means available for exploring such relationships, namely, the artificial association of tree seedlings with fungi in pure culture, was employed.

In earlier experiments (Melin, 1921) mycorrhizal fungi (*Mycelium radicis* subsp.) were isolated from tree roots and their relationships to tree seedlings demonstrated. By 1925 species among the genera *Lactarius, Russula, Cortinarius, Tricholoma, Amanita,* and *Boletus* had been shown to form mycorrhizaë with *Pinus, Picea, Larix, Betula,* or *Populus* (Melin, 1922, 1923a, 1923b, 1924, 1925a, 1925b, 1925c). Hammarlund (1923) and Masui (1927) have subsequently reported successful syntheses in pure culture.

Precise knowledge on the etiology of the mycorrhizal habit of American Pines is fundamental to studies of their nutrition.[1] With this larger problem in view, a series of syntheses experiments were pursued with *P. Strobus* L. and *P. resinosa* Ait. in association with species of *Boletinus, Boletus, Lactarius, Russula, Amanita,* and *Mycelium radicis* subsp. cultured in both Sweden and America (See Table 1). The study was initiated in Professor Melin's laboratory at the Mycological Laboratory, Royal Academy of Forestry, Stockholm, in the fall of 1929.

Descriptions of technique used in syntheses experiments have been published by Melin (1921, 1923a, 1925a). But Melin has been so frequently misquoted and his technique so often incorrectly followed that recapitulation in connection with the present work is desirable.

Cultures of known fungi were obtained by means of tissue isolations from the pileus and upper portion of the stipes of young sporophores. The following fungi were obtained in culture:[2]

[1] Absorbing roots (short roots, "Saugwurzeln") of Pines in natural habitats are completely mycorrhizal. Thus nutrients entering the tree by these channels must first pass through a fungal mantle that entirely separates the root cells of the tree from the soil.

[2] *Boletinus porosus* and *Boletus castaneus* were identified by Mr. C. L. Krieger, Washington, D. C., *Lactarius chrysorheus* by the junior author and all other specimens by Professors E. Melin and T. Lagerberg.

Lactarius chrysorheus Fr.
" *deliciosus* (L.) Fr., Plate 68, G.
" *subdulcis* (Bull.) Fr.
Russula fragilis (Pers.) Sing.
" *puellaris* Fr.
Amanita muscaria (L.) Fr., Plate 68, D.
Boletus chrysenteron (Bull.) Fr.
" *piperatus* Bull.
" *granulatus* L., Plate 68, B.
" *luteus* (L.) Fr.
" *castaneus* Bull., Plate 68, F.
" *bovinus* (L.) Fr., Plate 68, E.
" *edulis* Bull.
Boletinus porosus (Berk.) Peck, Plate 68, A.

The plan of the work included syntheses experiments with *Mycelium radicis* subsp. isolated from both American and Swedish conifers. The technique developed by Melin (1921, 1925a) for isolating mycorrhizal fungi was employed. Essentially this consists of: (1) selecting comparatively young and clean long-roots bearing mycorrhizae; (2) washing these thoroughly in a strong stream of tap-water; (3) cutting the roots into short lengths, each bearing one mycorrhiza; (4) surface sterilizing the latter in 0.1 per cent bichloride of mercury; and (5) rinsing in three or more changes of sterile water. The time required for surface sterilizing small mycorrhizae of the forked (*Pinus*) or racemose (*Picea*) types was two to five seconds (Melin, 1923a, p. 125). A considerable number of contaminations were inevitable, but longer treatments are lethal to the true endophytes. For tuberous mycorrhizae of Pine one minute usually did not prove injurious. After rinsing, the pieces were placed either in agar petri dishes or on nutrient liquid or agar in test tubes. Uncontaminated pieces were later transferred to suitable culture media.

All of the culture media employed contained, or consisted of, malt extract (See Melin, 1925a, p. 10). For the more sensitive fungi (obtained from either known sporophores or from roots) 5 per cent malt extract, sterilized by passage through a Berkefeld filter, was a suitable medium. Rapidly growing forms (*Boletus bovinus*, etc.) were cultivated on autoclaved 5 per cent malt extract with 2 per cent agar (See Table 1). In the latter case a minimum pressure and period of sterilization was conducive to rapid growth of the organisms.[1]

[1]The American brands of malt extract experimented with were not suitable. They are apparently evaporated at high temperatures, which destroy some nutritive properties. We have used Liebig's malt extract obtained from Apoteksvarucentral Vitrum, Stockholm.

TABLE 1

DATA ON FUNGAL CULTURES

Fungus	Place collected	Date	Most abundant trees in stand	Diam. growth of colonies in mm.	Color of hyphae	Color change of media	Type of Colony	Remarks
Lactarius chrysorheus	Westtown, Pa.	8/26/29	pure P. Strobus plantation	10–12 (ME)[c]	yellowish white	slight	loose, submerged	
Lactarius deliciosus	Djursholm[a]	9/22/29	Pinus, Betula	30–35 (ME)	yellow to whitish yellow	slight	loose, submerged (ME) aërial, submerged (MA) (Pl. 68)	Cultivated on solid media 4 mos. after isolation. Formed strands in culture with Pine.
Lactarius subdulcis	Djursholm	9/20/29	Pinus, Picea	3–4 (ME)	white	slight	compact, submerged	
Russula fragilis	Djursholm	9/20/29	Pinus, Picea	2–4 (ME)	white	slight	compact, submerged	
Russula puellaris	Djursholm	10/15/29	Betula, Pinus	2–4 (ME)	white	slight	compact, submerged	
Amanita muscaria	Djursholm	9/22/29	Betula	5–7 (MA)[f]	white	slight	compact, submerged (ME) compact, aërial (MA) (Pl. 68)	
Boletus chrysenteron	Experimentalfältet[a]	10/3/29	Quercus	2–4 (ME)	light brownish yellow	slight	compact, submerged	
Boletus piperatus	Djursholm	10/28/29	Pinus, Picea	8–10 (ME)	lemon yellow	dark brownish	loose, aërial	
Boletus granulatus	Djursholm	10/9/29	Pinus, Betula	50–55 (MA)	white, brownish with age	light brownish	loose, aërial with strands	Formed strands when associated with seedlings in pure culture.
Boletus luteus	Djursholm	9/20/29	Pinus, Betula	70–75 (MA)	white, brownish with age	dark brownish	aërial (Pl. 68)	
Boletus castaneus	Ansonia, Pa.	8/24/29	Tsuga, Betula	57–62 (MA)	white	pinkish	aërial even (Pl. 68)	

TABLE 1—Continued

DATA ON FUNGAL CULTURES

Fungus	Place collected	Date	Most abundant trees in stand	Diam. growth of colonies in mm.	Color of hyphae	Color change of media	Type of Colony	Remarks
Boletus bovinus	Djursholm	9/20/29	Pinus, Picea	65–70 (MA)	white, brownish with age	dark brownish	aërial (Pl. 68)	
Boletus edulis	Djursholm	9/20/29	Pinus, Betula	3–4 (ME)[c]	white	none	compact, submerged	
Boletinus porosus[d,e]	Warren, Pa.		Betula, Fagus, Tsuga, Prunus	64–69 (MA)[f]	deep brownish yellow	dark brown to black	crusted on surface of media, margin serrate	Numerous clamp connections
M.r. (Pinus) Strobi 1[e]	Ansonia, Pa.	Apr. '29	Conifer, hardwood	33–37 (MA)	white, brown with age	brownish	aërial	
M.r. (P.) sylvestris 1	Tureberg[a]	9/29/29	Pinus, Picea, Betula	35–40 (MA)	white, brown with age	brownish	aërial	
M.r. nigrostrigosum[b]	Kulbäcksliden[a]	4/2/30	Pinus, Betula, Picea		jet black	none	compact, submerged (ME) compact, aërial (MA)	
M.r. atrovirens 1[e]	Keene, N. H.	4/17/29	Pinus	85–95 (MA)	grayish green	none	See Melin 1923	
M.r. atrovirens 2[b]	Kulbäcksliden	4/2/30	Pinus, Betula, Picea	60–65 (MA)	grayish green	none	See Melin 1923	
M.r. (Picea) Abietis 1	Tureberg	9/29/29	Pinus, Picea, Betula	15–18 (ME) 10–12 (MA)	white-pinkish	brownish black	loose, submerged (ME)	Numerous clamp connections

a. Sweden.
b. Isolatcd from seedlings grown in soil-sand pot experiments by P. R. Gast. Soil from Brända Holmen, Kulbäcksliden Experimental Forest, Vindeln, Sweden.
c. ME—5 per cent liquid malt extract sterilized by passage through Berkefeld filter. MA—5 per cent malt extract, 2 per cent agar sterilized by autoclaving (Liebig's Malt Extract).
d. Isolated by A. H. Hough, Allegheny Forest Experiment Station. Subsequent isolations made by us in 1932 check exactly with original culture.
e. Cultured on an American brand of desiccated malt extract.
f. Those marked "(MA)" represent growth for 30 days on 30 ml. 5 per cent malt agar in 100 mm. Petri dishes.

In designating the imperfect stages of mycelia isolated from roots we have followed Burgeff (1932, p. 147) and included the generic names (in parenthesis) as well as the specific names of the vascular plants from which the mycelia were isolated. These names are of value to the individual investigator and likewise conveniently serve to inform the reader of the identities of the hosts from which they were isolated. In those cases where the mycelial characters are so marked that the fungus in question may easily be recognized by other investigators (particularly when the fungus is associated with more than one vascular plant), the name of the plant from which it was isolated is of less value and may be substituted by a descriptive specific name. Examples of such fungi are *Mycelium radicis atrovirens* Melin, and *M. r. nigrostrigosum* Hatch.

The following fungi were isolated from mycorrhizae of *Pinus Strobus*, *P. sylvestris* L., *Picea Abies* (L.) Karst.: *Mycelium radicis* (*Pinus*) *Strobi 1*, *M. r.* (*Pinus*) *sylvestris 1*, Plate 68, C, *M. r. ntgrostrigosum*, *M. r. atrovirens 1*, *M. r. atrovirens 2*, *M. r.* (*Picea*) *Abietis 1*, Plate 68, I.

The tree seeds used in our experiments were obtained through commercial seed houses. They were soaked over-night in water, surface sterilized for two minutes in 0.1 per cent bichloride of mercury, and rinsed in several changes of sterile water. They were then sown on agar in petri dishes, and contaminations and infected seeds were removed with a sterile spatula as they became evident. Early experience demonstrated that the particular sample of *P. Strobus* seeds we used required an after-ripening treatment to obtain even nominal germination. Barton (1928) had shown that cold storage treatment was effective in hastening germination of Southern Pine seeds. We, therefore, surface sterilized seeds of *P. Strobus* and stored them in a frigidaire at four to ten degrees centigrade for a period of two months. Regardless of the acidity of the media (moist filter paper, agar, and peat) which we varied from pH 3.5 to neutrality, germination was uniformly good (approximately 80 per cent).[1] Germination was procured in a constant temperature room at 25 degrees centigrade. As they germinated, the seeds were transferred directly into the culture chambers.

The culture technique developed by Melin (1925a, etc.) was employed in the syntheses. Fluvio-glacial sand was screened and that portion having particle sizes between 0.5 and 2.0 mm. was used (adequate aëration of the substratum is not possible in an undrained flask if smaller particles are included). The sand was boiled in concentrated

[1]Barton (1930) has published a second paper in which success is reported with low temperature treatments of *P. Strobus*.

hydrochloric acid for two hours, washed several hours in running tap-water, and finally in five changes of distilled water. It was dried in an oven, and 150 gram samples were weighed into 300 ml. Erlenmeyer flasks. The nutrient solution added to this substratum contained the following:

KH_2PO_4	0.5 grams
$CaCl_2$	0.05 "
NaCl	0.025 "
$MgSO_4 + 7\ H_2O$	0.15 "
$(NH_4)_2PO_4$	0.25 "
Iron citrate	0.025 "
Dextrose	0.5 "
Distilled water	1. litre

Thirty-seven ml. of this solution were added to each flask. The pH of the solution was 6.57. After autoclaving with the sand, this changed to approximately 4.2.

The nutrient conditions in our experiments differed from those in Melin's (1923a, p. 159). We used a less concentrated solution and likewise added 37 rather than 50 ml. of solution to each flask. Further, the growth of *P. Strobus* is considerably greater in culture than that of *P. sylvestris* seedlings. The differences in the nutritional conditions of the two sets of Pines were, therefore, quite marked. Since $\frac{\text{root}}{\text{shoot}}$ growth is greater when nutrients are present in comparatively small quantities, it was argued that more short roots would develop. The probability of obtaining mycorrhizae would therefore be enhanced. It is not necessary, however, to obtain large numbers of mycorrhizae in syntheses experiments, as has been emphasized by McArdle (1932, p. 314). The unquestionable demonstration of the presence of only one typical mycorrhiza is adequate proof that the organisms concerned enter into mycorrhizal association with each other.

The assembled culture chamber consisted of the Erlenmeyer flask with an inverted beaker over the cotton plug (Plate 70, D). Between March 23, 1930 and April 9, 54 of these units, each with a germinated seed of *P. Strobus*, and 52 units with *P. resinosa* seedlings, were set up. They were placed in the diffused light of a west window and received direct solar radiation, passing through the foliage of a large oak tree, late in the evening only (Plate 70, D). On June 5th to 7th the seedlings were inoculated with all of the fungi listed above. The subcultures from which inoculations were made were less than ten days old. A number of seedlings were reinoculated August 21.

Half of the flasks were opened during November 1930. These were

chiefly *P. Strobus* syntheses.[1] The remainder were kindly cared for by Professor Melin until the fall of 1931, when the seedlings were placed in fixing solution and shipped to the authors in America. Except for the latter, the substrata in all flasks were tested for contaminations at the close of the experiment by placing sand from the flasks on malt agar media in culture tubes. Of thirty-five cultures of *P. Strobus* opened in November 1930, three were contaminated. A number of contaminations from seed-coat infections with *P. resinosa* were observed during the course of the experiment. Similar contaminations with *P. Strobus* did not occur, since these seeds were on moist agar for nearly three months before they were transferred to the flasks and, consequently, seed-coat infections were eliminated.

Fixation was with Karpchinko solution; it did not prove particularly good for mycorrhizal details. The roots were embedded in paraffin for sectioning; both the butyl and ethyl alcohol series were used. Gross photographs of mycorrhizal roots were made in distilled water between glass plates. Microplanar or Tessar lenses, and Ilford panchromatic soft gradation plates with a Wratten B (red) filter were used. The staining technique employed will be reported by Dr. K. D. Doak in a future communication.

RESULTS

Typical ectotrophic mycorrhizae were form with *P. Strobus* by twelve of the fungi investigated:

Lactarius chrysorheus Fr.,	Plate 70, E
" *deliciosus* (L.) Fr.,	" 69, A; Plate 71, A
Amanita muscaria (L.) Fr.,	" 69, C; " 71, D
Boletus castaneus Bull.,	" 70, I
" *bovinus* (L.) Fr.,	" 70, F
" *luteus* (L.) Fr.,	" 69, B
" *granulatus* L.,	" 70, H
Boletinus porosus (Berk.) Peck,	" 70, G; Plate 71, C
Mycelium radicis nigrostrigosum	
" " (*Picea*) *Abietis* 1	Plate 71, B
" " (*Pinus*) *Strobi* 1	
" " (*Pinus*) *sylvestris* 1	

The mycelia of the *M. r. atrovirens* type, as in Melin's experiments, overgrew the aërial parts of the seedlings and failed to exhibit any indications of mycorrhiza-formation (Plate 70, A). The mycelia

[1] *P. resinosa* grew very poorly in the medium used in these experiments. Root development was adequate for mycorrhiza-formation in only two or three cases. These will be reported elsewhere.

of all other fungi failed to develop in the substrata, and information on their ability to form mycorrhizae was not obtained.

DISCUSSION

Positive results with *Boletinus porosus* adds *Boletinus* to the genera of fungi that have been proved to contain mycorrhiza-forming species. *Lactarius chrysorheus* and *Boletus castaneus* are also added to the list of known mycorrhizal organisms. The remaining fungi (with the exception of those isolated from tree roots) have previously been tested in pure culture with success as follows:

Lactarius deliciosus, with *Pinus mugo* Turra (*P. montana* Mill.), *P. sylvestris* and *Picea Abies* (Melin, 1924, 1925a).

Amanita muscaria, with *Betula pendula* Roth, *B. alba* Roth, *Larix decidua* Mill. (*L. europaea* DC.), *Pinus sylvestris,* and *Picea Abies* (Melin, 1923a, 1925a).

Boletus granulatus, with *P. sylvestris* and *P. mugo* (Melin, 1923a, 1924b).

Boletus bovinus, with *Pinus densiflora* Sieb. & Zucc. (Masui, 1927).

Boletus luteus, with *P. sylvestris, P. mugo, Larix decidua* Mill., *L. occidentalis* Nutt. and *Picea Abies* (Melin, 1923a, 1923b, 1925a).[1]

Concerning *Amanita muscaria,* Melin (1925a, p. 100) reports: "Der Pilz scheint aber den Pflanzen gegenüber eine ziemlich hohe Virulenz gehabt zu haben." A similar tendency was exhibited by our culture and by *Lactarius chrysorheus* in a 6-months synthesis as exemplified by the development of a heavy intercellular net. A culture plant removed after 15 months' association with *A. muscaria,* on the other hand, showed normal infection (Plate 69, C).

Concerning *Boletus bovinus,* Masui (1927) inoculated seedlings of *Pinus densiflora* growing in large test tubes on nutrient agar and reports that the seedlings were killed by this fungus. In Erlenmeyer flasks the fungus grew up the stem of the seedlings (l. c., p. 203 and Plate XI., Fig. 7). We note that in the latter photograph the plugs of the flasks

[1]Masui (1927) conducted syntheses experiments between *Pinus Thunbergii* Parl., *Quercus myrsinaefolia* Bl., *Q. phillyraeoides* Gray, *Q. glauca* Thunb., *Q. mongolica* Fisch. var. *grosseserrata* Rehd. & Wils., *Q. paucidentata* Franch., and a fungus which he isolated from the tuberous (compound) mycorrhizae of the last named Oak. He believed these mycorrhizae were formed by the mycelium of a *Boletus,* the sporophore of which was attached to the mycorrhiza from which isolations were made. He listed this sporophore as *Boletus luteus* (?). In a footnote (1927, p. 195) Masui mentions that Dr. Krieger believes the specimen may be *B. granulatus.* The identity of the culture is, therefore, not certain, and we have excluded these results from the list above, which represents authentic pure culture syntheses only.

were covered. The explanation of the behavior of *B. bovinus* as reported by Masui is, therefore, probably attributable to excessively high humidities. Our culture of *B. bovinus* grew very rapidly, and quickly covered the substrata within the flasks (the only fungus that covered the sand with surface growth), but exhibited no tendency to overgrow the seedlings (Plate 70, C). The mycorrhizae formed by *B. bovinus* possessed hyphal mantles one-fourth of their total thickness.

It is of interest to record here that in some of the cultures two easily separable fungi, *Mycelium radicis nigrostrigosum* and *M. r.* (*Picea*) *Abietis 1,* were associated with the same seedling. Typical mycorrhizae were formed by each of the fungi with short-roots, and in addition *M. r. nigrostrigosum* formed a secondary mantle over several of the mycorrhizae of the *M. r.* (*Picea*) *Abietis* type (Plate 71, B).

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EXPLANATION OF PLATES

Plate 68. Colonies of mycorrhiza-forming fungi grown in 100 mm. Petri dishes on 30 ml. of 5 per cent malt extract (Liebig's) agar for 30 days (60 days for D and 20 days for F) at 25 degrees centigrade, × 1.
A. *Boletinus porosus.*
B. *Boletus granulatus.*
C. *Mycelium radicis* (*Pinus*) *sylvestris 1.*
D. *Amanita muscaria.*
E. *Boletus bovinus.*
F. *Boletus castaneus.*
G. *Lactarius deliciosus.*
H. *Mycelium radicis* (*Picea*) *Abietis 1.*

Plate 69. Mycorrhizae on roots of *Pinus Strobus* seedlings grown in pure culture.
A. Whole root system of seedling inoculated with *Lactarius deliciosus.* All short roots mycorrhizal, × 4. (Photo, U. S. Dept. Agric.)
B. Mycorrhizae formed by *Boletus luteus,* × 9.
C. Mycorrhizae formed by *Amanita muscaria,* × 9.

Plate 70. A. Seedling of *Pinus Strobus* inoculated with *Mycelium radicis atrovirens 2,* showing mycelial growth on stem and lower leaves, × 2/3. (Top of flask removed for photo.)
B. Uninfected short roots of *P. Strobus.* Seventeen months old seedling, × 4.
C. Seedling of *P. resinosa* inoculated with *Boletus bovinus,* showing mycelial growth over sand substratum, × 2/3. (Top cut from flask for photo.)
D. Cultures of *P. Strobus* and *P. resinosa* in west window of the Royal Academy of Forestry, Stockholm.
E. Mycorrhizae of *P. Strobus* formed by *Lactarius chrysorheus,* × 6. (Photo, U. S. Dept. Agri.)
F. Mycorrhizal short roots of *P. Strobus* formed with *Boletus bovinus,* × 9.
G. Mycorrhizae of *P. Strobus* formed with *Bolctinus porosus,* × 8.

H. Mycorrhizal roots of *P. Strobus* formed with *Boletus granulatus,* × 6.
I. Mycorrhiza of *P. Strobus* formed with *Boletus castaneus,* × 10.

Plate 71. Photomicrographs of mycorrhizae of *P. Strobus* from syntheses experiments, showing distribution of mycelia between the cortical cells, and mantle structure.
A. Inoculum—*Lactarius deliciosus,* medial, longitudinal section, × 400. (Photomicrograph by K. D. Doak.)
B. Inocula—*Mycelium radicis* (*Picea*) *Abietis 1* and *M. r. nigrostrigosum,* somewhat oblique, longitudinal section, × 400. *M. r. nigrostrigosum* forming a secondary mantle over that of *M. r.* (*P.*) *Abietis 1.* (Photomicrograph by K. D. Doak.)
C. Inoculum—*Boletinus porosus,* medial, longitudinal section, × 370.
D. Inoculum—*Amanita muscaria,* oblique, transverse section, × 460. From 17-months old seedling.

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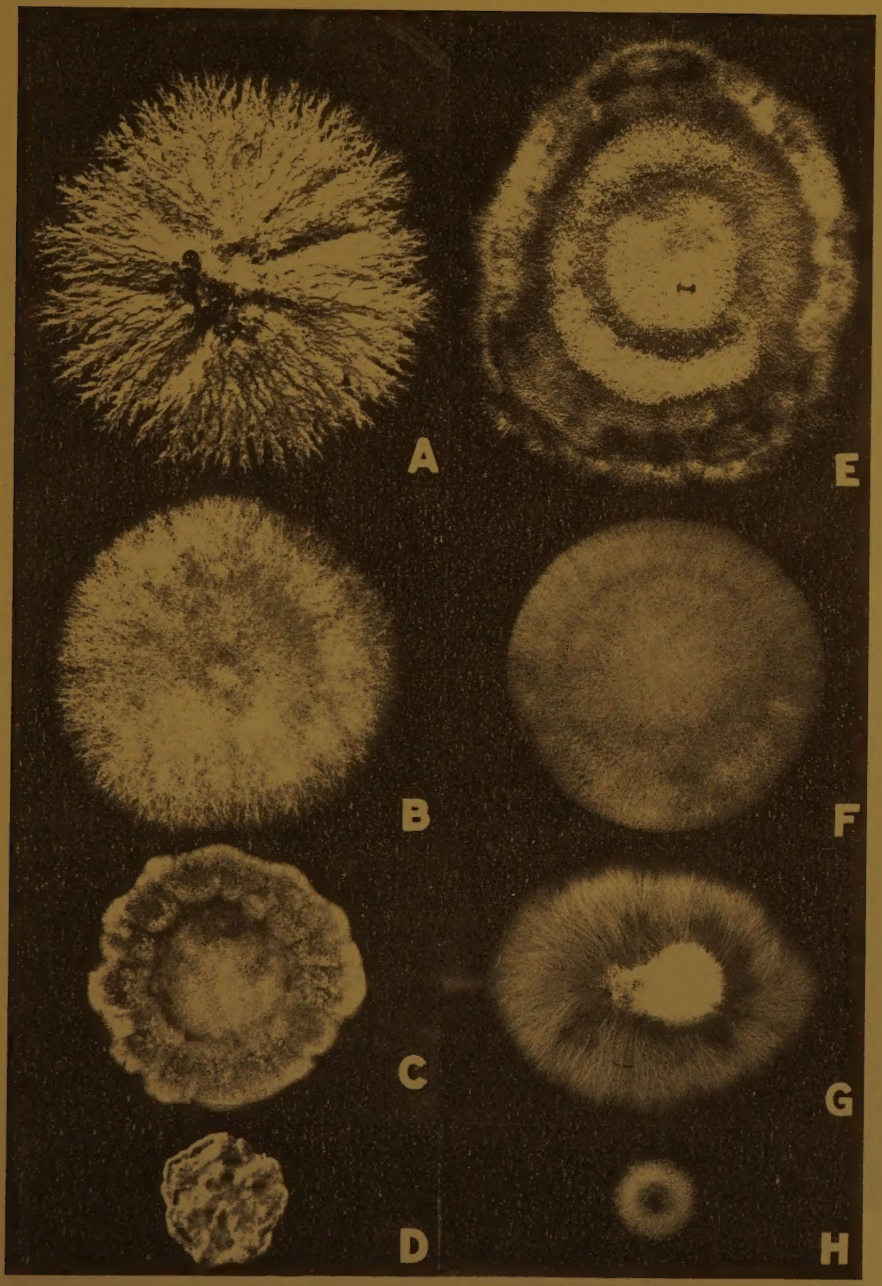
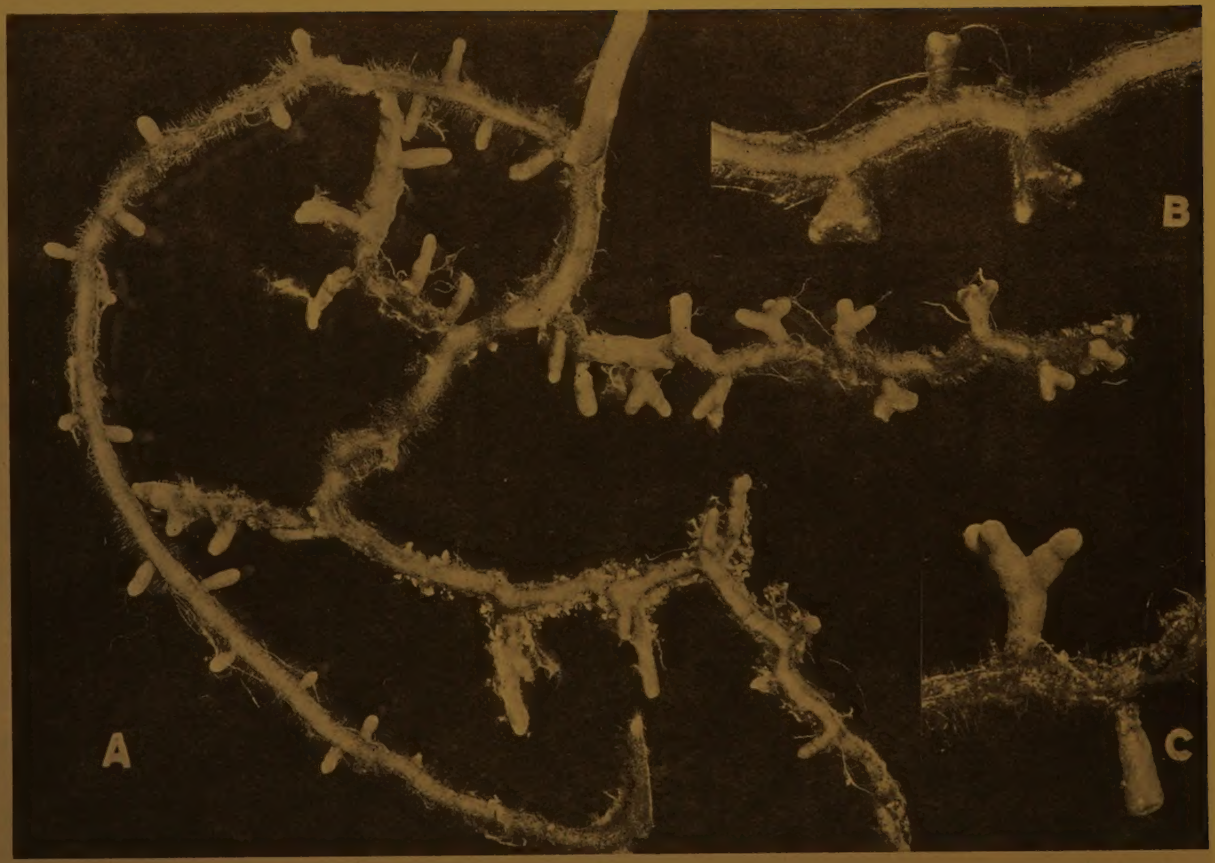


Some Hymenomycetes Forming Mycorrhizae with Pinus Strobus L.



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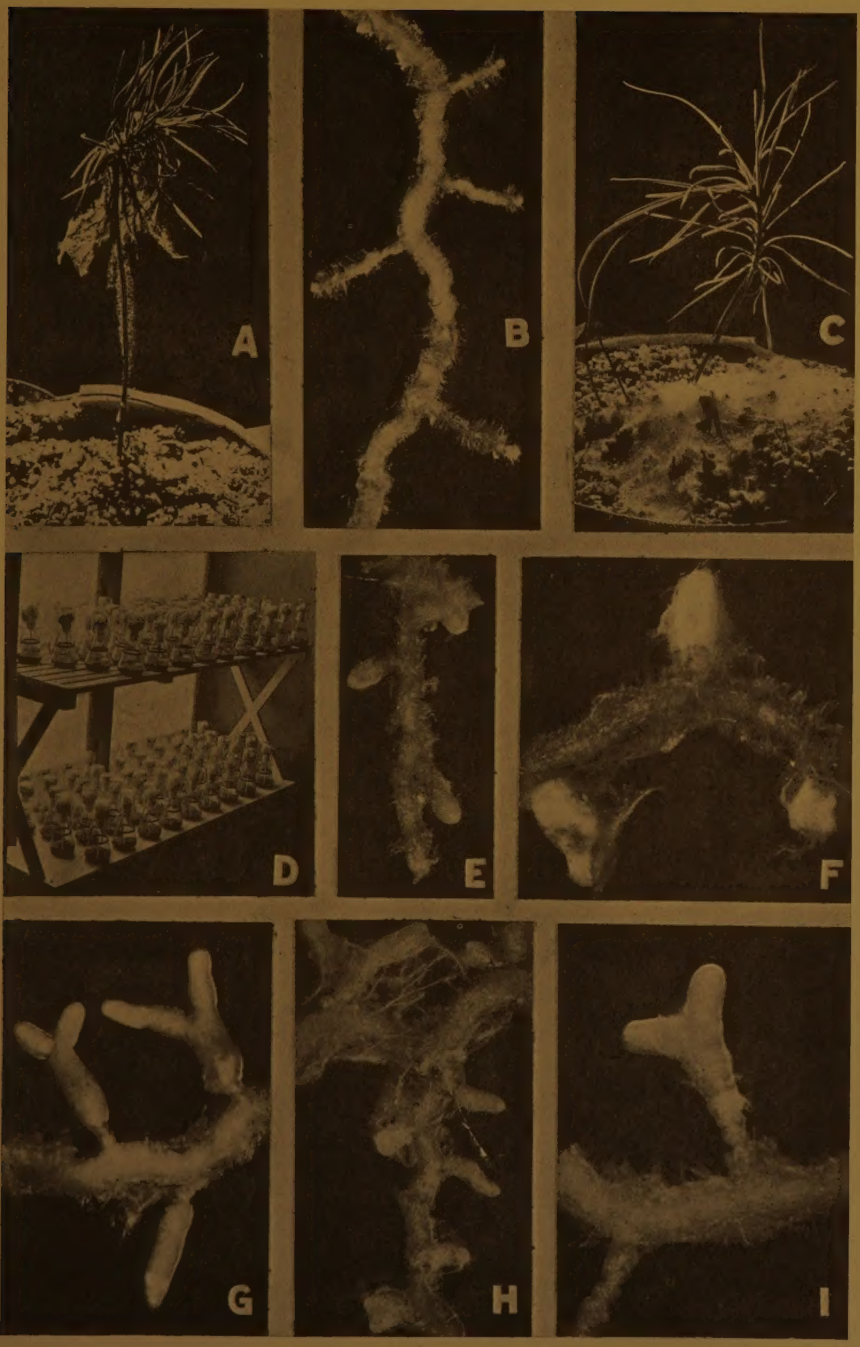

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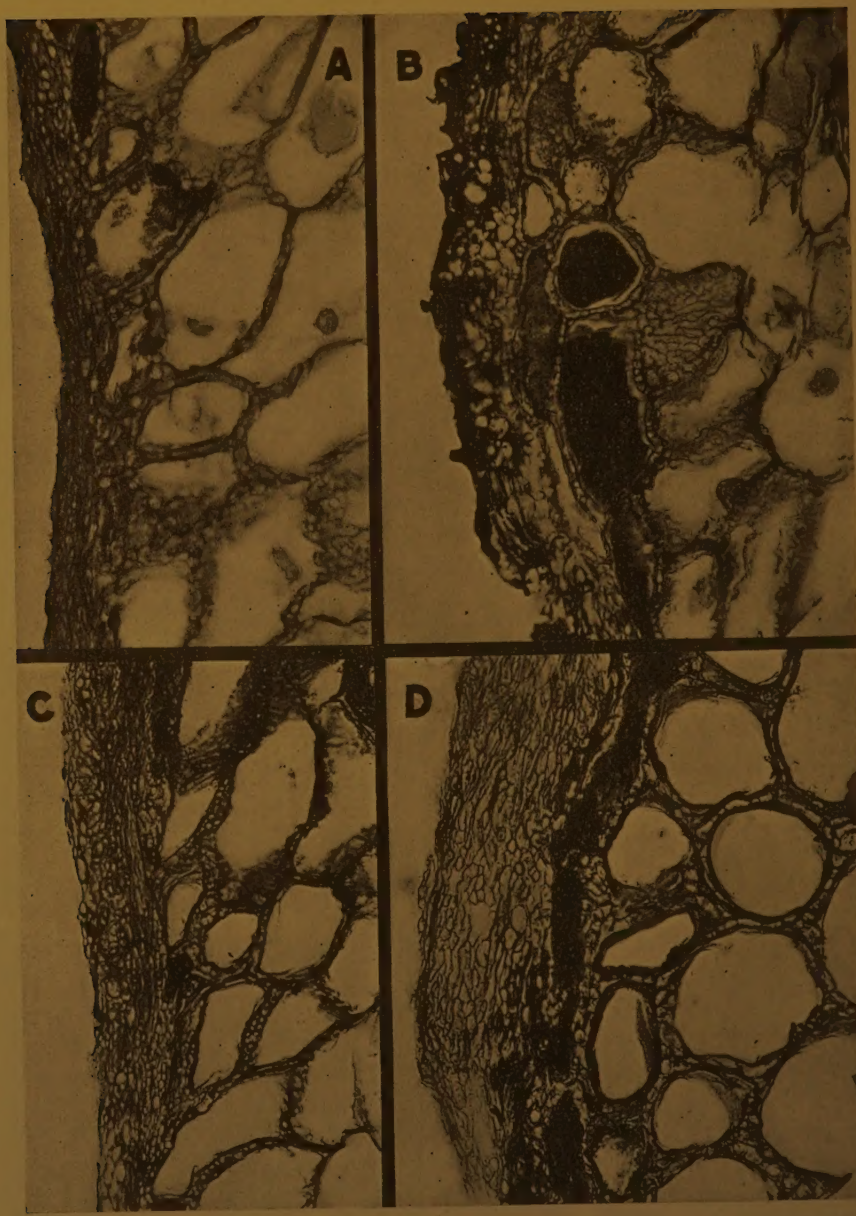

SOME HYMENOMYCETES FORMING MYCORRHIZAE WITH PINUS STROBUS L.